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Please find below and/or attached an Office communication concerning this application or proceeding.

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/935,168
Filing Date: August 21, 2001
Appellant(s): WEST ET AL.

Howard Speight
For Appellant

SUPPLEMENTAL EXAMINER'S ANSWER

This revised Examiner's Answer is to correct two deficient item(s) that were identified during review by Appeal specialist. They are: improper electronic signature by Examiner and missing heading # 1.

This is in response to the appeal brief filed 1/31/07 appealing from the Office action mailed 1/30/06.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows: The rejection of claims 24, 28, 31 and 35 under 35 U.S.C. 102(b) as being anticipated by US Pat 5,162,430 (Nov 10, 1992; PTO 892) is hereby withdrawn in view of the argument filed 1/31/07.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Stryer et al, in Biochemistry, Third edition, W H Freeman Company, New York, pages 31-33, 1998.

Dinbergs et al, J Biol Chem 271(47): 29822-29, 1996.

Scott-Burden et al, J Cardiovasc Pharmacol 16 Suppl 4: S36-41, 1990.

WO96/27657	WIPO	9-1996
5,162430	RHEE	11-1992
5,935,849	SCHINSTINE	8-1999

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

1. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

2. Claims 24-35 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling only for a method for making a tissue engineering scaffold as set forth in claims 1-5, 8 and 9, **does not** reasonably provide enablement for a method for making a tissue engineering scaffold using any matrix-enhancing molecule, any matrix-enhancing molecule such as any TGF beta, any angiotensin II, any insulin like growth factor and ascorbic acid at any concentration sufficient to elicit production of any extracellular matrix by any cell, any cell such as smooth muscle cells, endothelial cells, fibroblasts, chondrocytes, and any combination thereof attached to any engineering scaffold without increasing cellular proliferation of the attached cells as set forth in claims 24-35. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in **scope** with these claims.

Factors to be considered in determining whether undue experimentation is required to practice the claimed invention are summarized *In re Wands* (858 F2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). The factors most relevant to this rejection are the scope of the claim, the amount of direction or guidance provided, the lack of sufficient working examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention. The specification disclosure is insufficient to enable one skilled in the art to practice the invention as broadly claimed without an undue amount of experimentation.

The specification discloses only one member of the TGFβ family as the matrix-enhancing materials for inducing collagen production. The specification discloses the optimal concentrations to induce ECM production is in the range of between one and five ng TGF-β/ml for aortic smooth muscle cells, see page and between 5 and 100 ng TGFβ for chondrocytes, which is equivalent to between 4 x 10^{-6} and 4x 10^{-3} nmol/ml, see page 7, lines 11-20. The TGF-β is tethered or conjugated to polymer via polyethylene glycol (PEG), see page 9, last paragraph. The specification further discloses SMCs were then homogeneously seeded into polyethylene glycol (PEG) hydrogels containing covalently tethered RGDS (SEQ ID NO: 1). The hydrogels contained either no TGFβ, unmodified (soluble) TGFβ, or PEG-tethered TGFβ. In these photopolymerized hydrogels, the tethered peptides of TGFβ are covalently bound to the hydrogel structure via a highly flexible PEG chain. This gives the tethered moieties conformational freedom to interact with their receptors while causing them to be retained in the hydrogel material. After 7 days of culture, the hydrogels were digested and assayed for DNA and hydroxyproline. Since hydroxyproline is a marker for collagen, it is an indication of how much extracellular matrix has been produced. The results for cells grown in the presence of 0.04 pmol/ml (1 ng/ml) of TGFβ are presented in Figure 3. More hydroxyproline, and thus more collagen, was produced by SMCs grown in the presence of either soluble or tethered TGFβ than when no TGFβ was present. Additionally, significantly more hydroxyproline was produced when TGF-13 was tethered onto the hydrogels than when soluble TGF-β was used, see paragraph bridging page 13-14. For cell proliferation, aortic smooth muscle cells were grown on aminophase glass that had 0.5 nmol/cm2 RGDS (SEQ ID NO:l) covalently coupled to the glass. TGF-β was added to the media at 0, 1, or 5 ng/ml (0, 4 x 10^{5}, 2 x 10^{4} nmol/ml). ECM protein production by the cells over a 2-day time period rather than a 7-day time period was determined by examining the amount of 3H-glycine incorporated into the ECM elaborated by the cells. As seen in Figure 4, ECM protein production per cell (% of control) was increased when TGF-β was added to the media at both 1 and 5 ng/ml. Further, cell numbers did not increase over the 2 days, despite changes in matrix production per cell, see page 14 Example 2. The specification also discloses increases matrix production in the presence of ascorbic acid by smooth muscle cell and chondrocyte. Aortic smooth muscle cells and auricular chondrocytes were grown on tissue culture polystyrene with and without 50 μg/ml ascorbic acid added to the media (untethered ascorbic acid). ECM protein production by the cells over a 2-day time period was determined by examining the amount of 3H-glycine incorporated into the ECM elaborated by the cells. As seen

in Figure 7, ECM protein production per cell was increased in the presence of ascorbic acid for both SMCs (light gray) and chondrocytes (dark gray). Further, cell numbers did not increase over the 2 days (Figure 8).

The specification does not teach how to make all "matrix-enhancing molecule" covalently coupled to a polymer tether for the claimed method without the amino acid sequence of "matrix-enhancing molecule". Given the unlimited number of matrix enhancing molecules, there is insufficient guidance as to the structure and which matrix enhancing molecules would induce the production of which extracellular matrix by which cell type without increasing cellular proliferation of the attached cells to the scaffold, let alone at which particular concentration for the claimed method. Further, there is insufficient working example showing that other matrix enhancing molecule other than TGF-β is effective for inducing any matrix production in all cell type, in turn, would be useful for implantation. The specification does not teach how to predict which matrix-enhancing molecule is effective for inducing matrix production by which cell type.

Stryer et al teach that a protein is highly dependent on the overall structure of the protein itself and that the primary amino acid sequence determines the conformational of the protein (See enclosed appropriate pages).

The specification also discloses *the optimal density will depend on the type of cells to be attached to the scaffold*, see specification page 7, lines 11-20. In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng/ml for aortic smooth muscle cells and between 5 and 100 ng TGF- β per ml for chondrocytes, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng), see specification at page 15, Figure 7. Further, ascorbic acid was not tethered to the scaffold.

Given the unlimited number of matrix enhancing molecules and without the structure (i.e. chemical structure of amino acid sequence) and these matrix enhancing molecules have different effects on different cell type, it is unpredictable which undisclosed matrix-enhancing molecule and at which concentration is effective for inducing which matrix production for the claimed method with further guidance from the specification.

For these reasons, it would require undue experimentation of one skilled in the art to practice the claimed invention. See page 1338, footnote 7 of Ex parte Aggarwal, 23 USPQ2d 1334 (PTO Bd. Pat App. & Inter. 1992).

In re wands, 858 F.2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988), the decision of the court indicates that the more unpredictable the area is, the more specific enablement is necessary. In view of the quantity of experimentation necessary, the limited working examples, the unpredictability of the art, the lack of sufficient guidance in the specification and the breadth of the claims, it would take an undue amount of experimentation for one skilled in the art to practice the claimed invention.

3. Claims 24-35 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The specification does not reasonably provide a **written description** of any matrix-enhancing molecule, the concentration of any such matrix-enhancing molecule such as angiotensin II, insulin like growth factor other than TGF-β and ascorbic sufficient to elicit production of any extracellular matrix by any cell attached to any engineering scaffold.

The specification discloses only one member of the TGFβ family as the matrix-enhancing materials for inducing collagen production. The specification discloses the optimal concentrations to induce ECM production is in the range of between one and five ng TGF-β/ml for aortic smooth muscle cells, see page and between 5 and 100 ng TGFβ for chondrocytes, which is equivalent to between 4 x 10^{-6} and 4x 10^{-3} nmol/ml, see page 7, lines 11-20. The TGF-β is tethered or conjugated to polymer via polyethylene glycol (PEG), see page 9, last paragraph. The specification further discloses SMCs were then homogeneously seeded into polyethylene glycol (PEG) hydrogels containing covalently tethered RGDS (SEQ ID NO: 1). The hydrogels contained either no TGFβ, unmodified (soluble) TGFβ, or PEG-tethered TGFβ. In these photopolymerized hydrogels, the tethered peptides of TGFβ are covalently bound to the hydrogel structure via a highly flexible PEG chain. This gives the tethered moieties conformational freedom to interact with their receptors while causing them to be retained in the hydrogel material. After 7 days of culture, the hydrogels were digested and assayed for DNA and hydroxyproline. Since hydroxyproline is a marker for collagen, it is an indication of how much extracellular matrix has been produced. The results for cells grown in the presence of 0.04 pmol/ml (1 ng/ml) of TGFβ are presented in Figure 3. More hydroxyproline, and thus more collagen, was produced by SMCs grown in the presence of either soluble or tethered TGFβ than

when no TGFβ was present. Additionally, significantly more hydroxyproline was produced when TGF-13 was tethered onto the hydrogels than when soluble TGF-β was used, see paragraph bridging page 13-14. For cell proliferation, aortic smooth muscle cells were grown on aminophase glass that had 0.5 nmol/cm2 RGDS (SEQ ID NO:1) covalently coupled to the glass. TGF-β was added to the media at 0, 1, or 5 ng/ml (0, 4 x 10^5, 2 x 10^4 nmol/ml). ECM protein production by the cells over a 2-day time period rather than a 7-day time period was determined by examining the amount of 3H-glycine incorporated into the ECM elaborated by the cells. As seen in Figure 4, ECM protein production per cell (% of control) was increased when TGF-β was added to the media at both 1 and 5 ng/ml. Further, cell numbers did not increase over the 2 days, despite changes in matrix production per cell, see page 14 Example 2. The specification also discloses increases matrix production in the presence of ascorbic acid by smooth muscle cell and chondrocyte. Aortic smooth muscle cells and auricular chondrocytes were grown on tissue culture polystyrene with and without 50 μg/ml ascorbic acid added to the media (untethered ascorbic acid). ECM protein production by the cells over a 2-day time period was determined by examining the amount of 3H-glycine incorporated into the ECM elaborated by the cells. As seen in Figure 7, ECM protein production per cell was increased in the presence of ascorbic acid for both SMCs (light gray) and chondrocytes (dark gray). Further, cell numbers did not increase over the 2 days (Figure 8).

With the exception of one specific polypeptide matrix-enhancing molecule TGFβ tethered to polymer via PEG and one chemical matrix-enhancing molecule ascorbic acid that was not even tethered to polymer to eliciting collagen matrix production by smooth muscle cells and chrondocytes for the claimed method, there is insufficient written description about the structure associated with function of all other matrix-enhancing molecule, particularly the effective concentration effective to induce all matrix production without cell proliferation for all cells over any length of time for the claimed method. Given the unlimited number of matrix-enhancing molecule, the concentration effective for each undisclosed matrix-enhancing molecule for which cell type for the claimed method is not adequately described.

The specification discloses only one matrix enhancing polypeptide TGFbeta covalently coupled to PEG-diacrylate polymer, and one matrix enhancing chemical ascorbic acid that was not tethered to polymer for the claimed method, one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species of matrix-enhancing molecule and the concentration for all matrix enhancing molecule to describe the genus for the

claimed method. Thus, Applicant was not in possession of the claimed genus. *See University of California v. Eli Lilly and Co. 43 USPQ2d 1398; University of Rochester v. G.D. Searle & Co., 69 USPQ2d 1886 (CA FC2004).*

Applicant is directed to the Final Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 103(a) that form the basis for the rejections under this section made in this Office Action:

A person shall be entitled to a patent unless:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering Patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1-2, 4, 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892).

The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a matrix enhancing molecule such as TGF-β via a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The '430 patent also discloses other matrix enhancing molecule such as EGF, insulin like growth factor or combination thereof (see col. 6, line 63, in particular). The reference polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular).

The reference tissue engineering is useful for tissue or organ implantation or tissue regeneration (see col. 4, line 28-40, in particular). The '430 patent also teaches the concentration of various growth factor is preferably at a concentration of about 1μg/ml to about 5 mg/ml (see col. 10, lines 63-65, in particular). The '430 patent teaches by tethering factor molecules to the scaffold for implant, the effective amount of the growth factor is substantially reduced (see col. 7, lines 13-10, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired are easily be determined by routine experimentation (see col. 13, lines 11-17, in particular).

The claimed invention in claim 1 differs from the reference only in that the method wherein the TGF-beta is present at a density of between 1 and 100 ng/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold such as alginate/heparin-sepharose microsphere for inducing formation of extracellular matrix by cells such as endothelial cells and smooth muscle cells bound to said scaffold comprising coupling various matrix-enhancing molecule such as bFGF or TGF-β in a concentration 1-10 ng/ml (See Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The reference TGFβ is effective to elicit production of extracellular matrix (see page 29822, column 2, last paragraph, in particular) without increasing cellular proliferation (See Fig 2B, Fig 3B, Abstract, in particular). Dinbergs *et al* teach TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use TGF-β a concentration 1-10 ng/ml as taught by Dinbergs *et al* and tethered the TGF-β to a scaffold for a method of making a tissue engineering scaffold comprising TGF is covalently coupled to collagen or alginate via a polymer tethered as taught by the '430 patent and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Dinbergs *et al* teach TGFβ can be incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). One having ordinary skill in the art would have been motivated to tethered the growth factor to the scaffold because significantly less growth is needed as taught by the '430 patent (see col. 7, lines 13-10, in particular) and the tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

7. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Dinbergs et al (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892) as applied to claims 1-2, 4, 8 and 9 and further in view of Scott-Burden et al (of record, J Cardiovasc Pharmacol 16 Suppl 4: S36-41, 1990; PTO 892).

The combined teachings of the '430 patent and Dinbergs et al have been discussed supra.

The claimed invention in claim 3 differs from the teachings of the references only in that the method wherein the matrix-enhancing molecule is angiotensin II.

Scott-Burden et al teach the problem of increasing smooth muscle cell proliferation is that this proliferative activities may lead to the structural changes associated with hypertension and atherosclerosis that in themselves further stimulate the proliferative behavior of smooth muscle cells, see page S96, paragraph bridging col. 1 and 2, in particular). In fact, the specification discloses increased proliferation of smooth muscle cells could lead to narrowing of a vessel lumen, at page 3, line 17-18.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the TGF beta as taught by the '430 patent and Dinbergs et al for the angiotensin II as taught by Scott-Burden et al for a method of for making a tissue engineering scaffold for inducing formation of extracellular matrix by cells such as smooth muscle cell where the angiotensin II is covalently coupled to collagen via a polymer tethered such as PEG as taught by the '430 patent. From the combined teachings of the references, it is apparent that one of

ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because angiotensin II activates the synthesis of extracellular matrix such as glycopeptides and proteoglycans by smooth muscle cells as well as the growth of smooth muscle cell as taught by Scott-Burden et al (see abstract, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

8. Claims 5, 7 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892) as applied to claims 1-2, 4, 8 and 9 and further in view of US Pat No. 5,935,849 (or record, Aug 10, 1999; PTO 892).

The combined teachings of the '430 patent and Dinbergs et al have been discussed supra.

The claimed invention in claim 5 differs from the teachings of the references only in that the method wherein the matrix-enhancing molecule is ascorbic acid.

The claimed invention in claim 7 differs from the teachings of the references only in that the method wherein the scaffold is a hydrogel.

The claimed invention in claim 8 differs from the references only in that the method wherein the scaffold hydrogel is alginate and combination thereof.

The '849 patent teaches a method of making a tissue engineering scaffold such as bioartificial organ (BAO) using scaffold such as hydrogel or alginate or collagen (see col. 19, lines 22, col. 20, lines 42-36, summary of invention, in particular) covalently coupling to an inner matrix by a tether such as poly-d-lysine (see col. 18, line 30-35, in particular) coupling to matrix enhancing molecule such as RGD containing sequence (see col. 18, lines 36-51, in particular) or TGF beta and/or ascorbic acid (see col. 12, line 56-67, in particular). The '849 patent teaches TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells while ascorbic acid and TGFbeta1 increase collagen biosynthesis (see col. 12, lines 57-67, Table 1, in particular). The reference method further comprises providing cells such as fibroblast or endothelial cells attached within the tissue-

engineering scaffold (see col. 16, Table 1, Col. 19, line 29, in particular). The reference method is useful for implantation and controlling distribution of cells within the bioartificial organ (see claims of '849 patent).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the collagen and TGF beta in the tissue engineering scaffold comprising collagen covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix enhancing molecule such as TGFbeta as taught by the '430 patent for the hydrogel such as alginate and ascorbic acid as taught by the '849 patent for a method of making a tissue engineering scaffold comprising the hydrogel such as alginate covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix-enhancing molecule such as TGFbeta and/or ascorbic acid as taught by the '430 patent, Dinbergs et al and the '849 patent. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells, while ascorbic acid and TGFbeta1 increase collagen biosynthesis as taught by the '849 patent (see col. 12, lines 57-67, Table 1, in particular). The use of engineering scaffold is useful to control cell number, cell distribution and attachment in organ transplant as taught by the '849 patent. Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

9. Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892) as applied to claims 1-2, 4, 8 and 9 and further in view of WO 94/23740 (of record, Oct 1994, PTO 1449) or WO 96/27657 (Sept 1996; PTO 1449).

The combined teachings of the '430 patent and Dinbergs et al have been discussed supra.

The claimed invention in claim 8 differs from the teachings of the references only in that the method wherein the scaffold is a hyaluronic acid or polyethylene glycol polymer instead of collagen.

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 covalently coupling to polyethylene glycol (See page 12, line 11, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication teaches the method of making a tissue engineering scaffold comprising coupling TGFβ to a polymer is useful for stimulation of bone formation at a lower dose (See abstract, in particular).

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) covalently coupled to a scaffold such as hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate, (See page 17, line 8, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the collagen and TGF beta in the tissue engineering scaffold comprising collagen covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix enhancing molecule such as TGFbeta as taught by the '430 patent for the polyethylene glycol as taught by the WO 94/23740 publication or the hyaluronic acid as taught by the WO 96/27657 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because polyethylene glycol covalently to TGFβ2 is useful for stimulation of bone formation at a lower dose as taught by the WO 94/23740 publication (See abstract, in particular). The WO 96/27657 publication teaches hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate coupled to TGFβ is useful for localized the desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

10. Claims 24-27 and 32-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of US Pat No. 5,935,849 (of record, Aug 10, 1999; PTO 892).

The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a matrix enhancing molecule such as TGF-β via a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The '430 patent also discloses other matrix enhancing molecule such as EGF, insulin like growth factor or combination thereof (see col. 6, line 63, in particular). The reference polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The reference tissue engineering is useful for tissue or organ implantation or tissue regeneration (see col. 4, line 28-40, in particular). The '430 patent also teaches the concentration of various growth factor is preferably at a concentration of about 1μg/ml to about 5 mg/ml (see col. 10, lines 63-65, in particular). The '430 patent teaches by tethering factor molecules to the scaffold for implant, the effective amount of the growth factor is substantially reduced (see col. 7, lines 13-10, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired are easily be determined by routine experimentation (see col. 13, lines 11-17, in particular).

The claimed invention in claim 25 differs from the teachings of the reference only in that the method further comprises providing cell attached to the tissue engineering scaffold.

The claimed invention in claim 26 differs from the teachings of the reference only in that the method further comprises providing cell attached to the tissue engineering scaffold wherein the cell is attached within the scaffold.

The claimed invention in claim 27 differs from the teachings of the reference only in that the method wherein the cell is selected from the group consisting of endothelial cells, fibroblasts, and combination thereof.

The claimed invention in claim 32 differs from the teachings of the reference only in that the method wherein the matrix enhancing molecule is ascorbic acid.

The claimed invention in claim 33 differs from the teachings of the reference only in that the method wherein the scaffold is a hydrogel.

The claimed invention in claim 33 differs from the teachings of the reference only in that the method wherein the scaffold hydrogel is alginate, hyaluronic acid, polyethylene glycol polymers and combination thereof.

The '849 patent teaches a method of making a tissue engineering scaffold such as bioartificial organ (BAO) using scaffold such as hydrogel or alginate or collagen (see col. 19, lines 22, col. 20, lines 42-36, summary of invention, in particular) covalently coupling to an inner matrix by a tether such as poly-d-lysine (see col. 18, line 30-35, in particular) coupling to matrix enhancing molecule such as RGD containing sequence (see col. 18, lines 36-51, in particular) or TGF-β and/or ascorbic acid (see col. 12, line 56-67, in particular). The '849 patent teaches TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells while ascorbic acid and TGFbeta1 increase collagen biosynthesis (see col. 12, lines 57-67, Table 1, in particular). The reference method further comprises providing cells such as fibroblast or endothelial cells attached within the tissue engineering scaffold (see col. 16, Table 1, Col. 19, line 29, in particular). The reference method is useful for implantation and controlling distribution of cells within the bioartificial organ (see claims of '849 patent).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute (1) the collagen taught by the '430 patent for the hydrogel such as alginate as taught by the '849 patent and (2) the TGF-β taught by the '430 patent for the TGF-β and/or ascorbic acid as taught by the '849 patent for a method of making a tissue engineering scaffold comprising the hydrogel such as alginate covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix-enhancing molecule TGFbeta and/or ascorbic acid as taught by the '430 patent and the '849 patent. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells, while ascorbic acid and TGFbeta1 increase collagen biosynthesis as taught by the '849 patent (see col. 12, lines 57-67, Table 1, in particular). The use of engineering scaffold is useful for controlling the cell number, the cell distribution and attachment in organ transplant as taught by the '849 patent. The method of tissue engineering is

useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

11. Claims 27 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of US Pat No 5,935,849 (of record, Aug 10, 1999; PTO 892) as applied to claims 24-27 and 32-34 mentioned above and further in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892).

The combined teachings of the '430 patent and the '849 patent have been discussed supra.

The claimed invention in claim 27 differs from the references only in that the method wherein the cell is smooth muscle cells.

The claimed invention in claim 29 differs from the references only in that the method wherein the TGF-beta is present at a density of between 1 and 100 ng/ml or in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold such as alginate/heparin-sepharose microsphere for inducing formation of extracellular matrix by cells such as endothelial cells and smooth muscle cells bound to said scaffold comprising coupling various matrix-enhancing molecule such as bFGF or TGFβ in a concentration 1-10 ng/ml (See Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The reference TGFβ is effective to elicit production of extracellular matrix (see page 29822, column 2, last paragraph, in particular) without increasing cellular proliferation (See Fig 2B, Fig 3B, Abstract, in particular). Dinbergs *et al* teach TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute fibroblast as taught by the '849 patent for the smooth muscle cell or endothelial cells and matrix enhancing molecule TGFbeta at concentration 1-10 ng/ml as taught by Dinbergs *et al* for a method of for making a tissue engineering scaffold for inducing

formation of extracellular matrix by cells such as smooth muscle cell or endothelial cells where the TGF is covalently coupled to collagen or alginate via a polymer tethered as taught by the '430 patent, the '849 patent and Dinbergs *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Dinbergs *et al* teach TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular) and that TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The use of engineering scaffold is useful for controlling the cell number, the cell distribution and attachment in organ transplant as taught by the '849 patent. The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

12. Claims 24 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Scott-Burden et al (of record, J Cardiovasc Pharmacol 16 Suppl 4: S36-41, 1990; PTO 892).

The teachings of the '430 patent have been discussed supra.

The claimed invention in claim 30 differs from the teachings of the reference only in that the method wherein the matrix-enhancing molecule is angiotensin II instead of TGF beta.

Scott-Burden et al teach the problem of increasing smooth muscle cell proliferation is that this proliferative activities may lead to the structural changes associated with hypertension and atherosclerosis that in themselves further stimulate the proliferative behavior of smooth muscle cells, see page S96, paragraph bridging col. 1 and 2, in particular). In fact, the specification discloses increased proliferation of smooth muscle cells could lead to narrowing of a vessel lumen, at page 3, line 17-18.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute TGF-β as taught by the '430 patent for the angiotensin II as taught by Scott-Burden et al for a method of for making a tissue engineering scaffold for inducing formation of extracellular matrix by cells such as smooth muscle cell where the angiotensin II is

covalently coupled to collagen or alginate via a polymer tethered as taught by the '430 patent, the '849 patent and Scott-Burden et al. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because angiotensin II activates the synthesis of extracellular matrix such as glycopeptides and proteoglycans by smooth muscle cells and growth of smooth muscle cell as taught by Scott-Burden et al (see abstract, in particular). The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

13. Claims 24 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of WO 94/23740 (of record, Oct 1994, PTO 1449) or WO 96/27657 (of record, Sept 1996; PTO 1449).

The teachings of the '430 patent have been discussed supra.

The claimed invention in claim 34 differs from the teachings of the reference only in that the method wherein the scaffold is hyaluronic acid or polyethylene glycol polymers.

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 covalently coupling to polyethylene glycol (See page 12, line 11, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication teaches the method of making a tissue engineering scaffold comprising coupling TGFβ to a polymer is useful for stimulation of bone formation at a lower dose (See abstract, in particular).

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) covalently coupled to a scaffold such as hyaluronic acid (see page 7, line 1, in particular) or collagen, or polyethylene oxide, or alginate, (See page 17, line 8, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the collagen and TGF beta in the tissue engineering scaffold

comprising collagen covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix enhancing molecule such as TGFbeta as taught by the '430 patent for the polyethylene glycol as taught by the WO 94/23740 publication or the hyaluronic acid or polyethylene oxide, or alginate as taught by the WO 96/27657 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because polyethylene glycol covalently to TGFβ2 is useful for stimulation of bone formation at a lower dose as taught by the WO 94/23740 publication (See abstract, in particular). The WO 96/27657 publication teaches hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate coupled to TGFβ is useful for localized the desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

(10) Response to Argument

Enablement Rejection for claims 24-35 under 35 U.S.C. 112, first paragraph

At page 8 of the Brief, Appellants argue that the Examiner has misconstrued the meaning of "matrix-enhancing molecules," and in so doing has incorrectly concluded that independent claim 24 is not enabled. Although Applicants have not explicitly defined the term "matrix-enhancing molecule" in the specification, the Examiner characterized the term as having been explicitly defined. Moreover, the Examiner's definition is incorrect. The Examiner stated, "The specification defines matrix-enhancing molecules at page 6 as any glycoproteins, any glycoproteins such as elastin, collagen, TGF-β, agniotensin II [sic], insulin-like growth factors, and ascorbic acid." Neither does the specification explicitly define the "scaffold materials", "tethers" nor "cells". By misconstruing the meaning of "matrix-enhancing molecule", the Examiner incorrectly concluded that independent claim 24 is not enabled.

Appellants' arguments have been fully considered but are not found to be persuasive. The specification discloses "Matrix-enhancing molecules which promote increased production of ECM can be attached to the scaffold material to induce production of matrix proteins, such as glycoproteins, elastin, and collagen, without substantially increasing cell proliferation. *These matrix-enhancing molecules include TGF--β, angiotensin II, insulin-like growth factors and*

ascorbic acid", see specification at page 6 lines 6-11. Further, the term "include" is open-ended. The matrix-enhancing molecules are not limited to TGF-β, angiotensin II, insulin-like growth factors and ascorbic acid as argued.

With respect to the argument of "tethers", the specification discloses "these tethers have a molecular weight of preferably between about 200 and 10,000, most preferably between about 2000 and 6,000. The tether is preferably a linear polymer, such as polyethylene glycol. The matrix-enhancing molecule may be coupled to the tether, or for that matter, to the scaffold material, by any method known to those of skill in the art, preferably covalently coupled using a reagent such as n-hydroxysuccinimide, carbodiimide, diisocyanate, carbonyldiamidazole, or tosyl chloride", see specification at page 7, lines 1-10.

With respect to the argument of "cell", the specification discloses the source of cells can be can be obtained directly from a donor, from a culture of cells from a donor, or from established cell culture lines. Preferred cells for formation of vascular tissue include smooth *muscle cells, endothelial cells, and fibroblasts*. Preferred cells for formation of connective tissue include chondrocytes, fibroblasts, and other types of cells that differentiate into bone or cartilage, see specification paragraph bridging pages 7 and 8.

At page 9-10 of the Brief, Appellants argue that because the Examiner's has misconstrued the meaning of "matrix-enhancing molecules," and in so doing has required that Applicants provide amino acid sequences that simply do not exist. A skilled artisan does not need to know the amino acid sequence of a particular matrix-enhancing molecule to make and use the claimed methods. In fact, matrix-enhancing molecules such as ascorbic acid do not even have an amino acid sequence. Furthermore, matrix-enhancing molecules are well known and well documented; there is a great deal of publicly available information about matrix-enhancing molecules. The art cited by the Examiner lists many matrix-enhancing molecules. See, e.g., '430 Patent, col. 6, 11. 55-66; '849 Patent, col. 16, Table 1. "The specification need not disclose what is well known to those skilled in the art and preferably omits that which is welt-known to those skilled and already available to the public. (citations omitted)." MPEP § 2164.05(a).

Appellants' arguments have been fully considered but are not found to be persuasive. As stated above, these matrix-enhancing molecules are limited to TGF-β, angiotensin II, insulin-like growth factors and ascorbic acid, see specification at page 6 lines 6-11 as argued. This is because the term "include" is used to define the term matrix-enhancing molecule. Other than the specific

matrix-enhancing molecules, there is insufficient guidance as to the structure, i.e. amino acid sequence of all matrix-enhancing molecules for the claimed method of making a tissue engineering scaffold.

Stryer et al teach that a protein is highly dependent on the overall structure of the protein itself and that the primary amino acid sequence determines the conformational of the protein (See enclosed appropriate pages).

The specification does not teach any assays that is useful for screening matrix enhancing molecule, much less predicting which undisclosed matrix-enhancing molecule at which concentration is sufficient to stimulate matrix production without inducing proliferation of which cell types.

Even if the matrix enhancing molecule is limited to TGF-β and without the concentration, the same TGF-β stimulates cell proliferation rather than reduces proliferation. See page 23 of the Brief, appellants argue that the '430 Patent discloses concentrations sufficient "to stimulate tissue growth to a detectable degree[, and] [t]issue, in this context, includes connective tissue, bone, cartilage, epidermis and dermis, blood, and other tissue." '430 Patent, col. 7, 11. 17-22. Thus, the '430 Patent teaches stimulating cell proliferation rather than reduces proliferation.

With respect to matrix production by TGF-β, the specification discloses the amount of ECM production that is most desirable is that which results in formation of tissue with good mechanical properties, on and within the tissue engineering scaffold. *The optimal density will depend on the type of cells to be attached to the scaffold.* In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng/ml for aortic smooth muscle cells and between 5 and 100 ng TGF- β per ml for chondrocytes, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng), see specification at page 15, Figure 7.

The scope of claim 24 is very broad. The specification describes the concentration for only two matrix-enhancing molecules having very different structures, namely TGF-β and ascorbic acid. Further, ascorbic acid was not tethered to any scaffold via any polymer tether.

Given the numerous matrix-enhancing molecules as encompassed by the claims, the specification provides little or no guidance as to concentration of other matrix enhancing molecule tethered to the scaffold, only one working example for TGF and one working example for chemical compound ascorbic acid, and coupled with the unpredictability in the art, it would

take undue amount of experimentation to enable one of skill in the art to practice the claimed invention.

At page 12 of the Brief, Appellants argue that Cell types are enabled. Regarding guidance for "which cell type," such is provided by Applicants' specification, as well as by what well known to those skilled in the art. First, Applicants' specification provides examples of various cell types, as well as sources for such cells. (Application p. 7, 1.21-p. 8, 1. 9.) And the use of various cell types is well known in the art, as the art cited by the Examiner shows. See, e.g., Dinbergs at 29825, col. 1-2 (discussing endothelial cells and smooth muscle cells); '849 Patent, col. 16, Table 1 (listing a number of cell types); WO 96/27657 at 14, 11. 14-27.

Appellants' arguments have been fully considered but are not found to be persuasive. The breadth of claim 24 is very broad. Claim 24 is drawn to a method for making a tissue engineering scaffold that encompassed providing any scaffold, any polymer tether, and any matrix-enhancing molecule; covalently coupling the polymer tether to the scaffold; and covalently coupling the matrix-enhancing molecule to the scaffold, wherein the matrix-enhancing molecule is present at any concentration sufficient to elicit production of extracellular matrix by any cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell.

The specification discloses the source of cells can be can be obtained directly from a donor, from a culture of cells from a donor, or from established cell culture lines. Preferred cells for formation of vascular tissue include *smooth muscle cells, endothelial cells, and fibroblasts*. Preferred cells for formation of connective tissue include chondrocytes, fibroblasts, and other types of cells that differentiate into bone or cartilage, see specification paragraph bridging pages 7 and 8. The specification also discloses *the optimal density will depend on the type of cells to be attached to the scaffold*, see specification page 7, lines 11-20. In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng/ml for aortic smooth muscle cells and between 5 and 100 ng TGF- β per ml for chondrocytes, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng) for *smooth muscle cells, endothelial cells*, see specification at page 15, Figure 7.

At page 13-14 of the Brief, Appellants argue that the claim language itself provides a functional concentration of the matrix-enhancing molecule that can be determined by routine testing without undue experimentation: "sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell." See MPEP 2164. The specification also describes that "[t]he optimal density [of the matrix-enhancing molecule] will depend on the type of cells to be attached to the scaffold." (Application at 7, 11. 11-20.). Appellants further argue that experimentation, if required is undue.

Appellants' arguments have been fully considered but are not found to be persuasive. The specification discloses concentration for *two* structurally unrelated matrix-enhancing molecule for the claimed method. In the case of TGF-β, the optimal concentrations to induce ECM production is in the range of between one and five ng/ml for aortic smooth muscle cells and between 5 and 100 ng TGF- β per ml for chondrocytes, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng), see specification at page 15, Figure 7. With respect to the argument the concentration effects different cell types, even the disclosure admitted that the concentration for a particular matrix-enhancing molecule "will depend on the type of cell to be attached to the scaffold.", see specification at page 7, lines 11-20. Given the unlimited number of matrix-enhancing molecule, the lack of guidance as to the structure of all matrix-enhancing molecule, the concentration of matrix-enhancing molecule other than TGF-β and ascorbic acid, the numerous cell types, the effect on such cells differs with respect to concentration of the matrix-enhancing molecule and cell types, the specification merely extends an invitation to one skill in the art to coming up with the structure of matrix-enhancing molecule and then further experimentation to determine which concentration is appropriate for the claimed method. Accordingly, it would require undue experimentation of one skilled in the art to practice the claimed invention. See page 1338, footnote 7 of Ex parte Aggarwal, 23 USPQ2d 1334 (PTO Bd. Pat App. & Inter. 1992).

Written Description Rejection for claims 24-35 under 35 U.S.C. 112, first paragraph

At pages 16-17 of the Brief, appellants argue that the specification adequately describes matrix-enhancing molecules. For example, the specification states that "[m]atrix-enhancing

molecules which promote increased production of ECM can be attached to the scaffold material to induce production of matrix proteins...without substantially increasing cell proliferation. These matrix-enhancing molecules include TGF-β, angiotensin II, insulin-like growth factors and ascorbic acid." (Application at 6, 11.7-11 .) The specification also discloses that the concentration for a specific matrix-enhancing molecule "will depend on the type of cell to be attached to the scaffold." (Application at 7, 11.15-16.) Moreover, Applicant's specification provides exemplary concentrations of matrix-enhancing molecules. For example, the concentration of the matrix-enhancing molecule TGF-β needed to elicit ECM production in auricular chondrocytes is provided. (Application at 7, 11.18-20.) As another example, the concentration of another specific matrix-enhancing molecule that is sufficient to produce ECM in aortic smooth muscle cells and auricular chondrocytes is also provided. (Application at 15, 11.4-14.) Furthermore, as originally filed, Applicants' independent claims 1, 10, and 16 specifically recite that the concentration of the matrix-enhancing molecule is "an effective density to elicit production of extracellular matrix without increasing cellular proliferation." (Application at 16-17, claims 1, 10, 16.) Applicants further note that the prior art cited by the Examiner shows that suitable matrix-enhancing molecules are well known. See, e.g., '430 Patent, col. 6, 11. 55-66; '849 Patent, col. 12, 11.57-62; col. 16, Table 1. In short, "martix-enhancing molecules" refers to a known class of molecules of known structure and function. One of ordinary skill in the art would perceive the claimed subject matter as having been possessed by Applicants. The Examiner fails to establish any evidence to conclude otherwise.

Appellants' arguments have been fully considered but are not found to be persuasive. It is noted that claims 1, 10 and 16 are not under rejection.

Claim 24 recites a method for making a tissue engineering scaffold, the method comprising: providing a scaffold, a polymer tether, and a matrix-enhancing molecule; covalently coupling the polymer tether to the scaffold; and covalently coupling the matrix-enhancing molecule to the scaffold, wherein the matrix-enhancing molecule is present at a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell.

Claim 24 encompassed any matrix-enhancing molecule at any concentration sufficient to elicit production of extracellular matrix by any cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell for the claimed method.

The specification discloses "Matrix-enhancing molecules which promote increased production of ECM can be attached to the scaffold material to induce production of matrix proteins, such as glycoproteins, elastin, and collagen, without substantially increasing cell proliferation. These matrix-enhancing molecules include TGF-β, angiotensin II, insulin-like growth factors and ascorbic acid", see specification at page 6 lines 6-11. Further, the term "include" is open-ended. The matrix-enhancing molecules are not limited to TGF-β, angiotensin II, insulin-like growth factors and ascorbic acid as argued. The specification also discloses that the concentration for a specific matrix-enhancing molecule "will depend on the type of cell to be attached to the scaffold.", see specification at 7, lines 15-20. The specification discloses the concentration of the two structurally different Matrix-enhancing molecules, namely TGF-β (protein) and ascorbic acid (chemical). For TGF-β, the optimal concentrations to induce ECM production is in the range of between one and five ng/ml for aortic smooth muscle cells and between 5 and 100 ng TGF- β per ml for chondrocytes, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng), see specification at page 15, Figure 7.

The specification dose not describe the structure of all matrix-enhancing molecule, much less the concentration of such matrix-enhancing molecule sufficient to elicit production of extracellular matrix by all cell attached to the tissue engineering scaffold without increasing cellular proliferation of such attached cell.

The specification discloses only one polypeptide TGF-β and one chemical compound ascorbic acid as the matrix-enhancing molecule to stimulate extracellular matrix production as measured by ^{3H}-glycine incorporation into the ECM elaborated by the cells, one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species of matrix-enhancing molecule to describe the genus for the claimed method. Thus, Applicant was not in possession of the claimed genus. *See University of California v. Eli Lilly and Co. 43 USPQ2d 1398; University of Rochester v. G.D. Searle & Co., 69 USPQ2d 1886 (CA FC2004).*

Applicant is directed to the Final Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

At page 17 of the Brief, appellants argue that the specification adequately describes extracellular matrix. Extracellular matrix is well-known in the art as evidenced by the references of record. Moreover, Applicants' specification sufficiently describes extracellular matrix (ECM). Applicants' specification states that ECM includes "matrix proteins, such as glycoproteins, elastin, and collagen." (Application at 6, 11.8-9.) The specification explains that "[i]n order to maintain proper mechanical integrity of the tissue, the cells must generate sufficient extracellular matrix (ECM)." (Application at 1, 11. 17-19.) Furthermore, the specification describes how to evaluate matrix protein production, including the composition of the ECM. (Application 10, 11. 1-26; ld. at 12, 11. 1-5.) In addition to the disclosure provided by Applicants' specification, persons skilled in the art recognize that the extracellular matrix is well characterized, as the art cited by the Examiner shows. See, e.g., Dinbergs at 29826-27, Discussion and references cited therein; Scott-Burden at $36, col. 2 and references cited therein; '849 Patent, col. 14, 11. 31-39, col. 15, 3-col. 16, 1.46; WO 96/27657 at 11, 1I. 11-13.

Appellants' arguments have been fully considered but are not found to be persuasive. The specification states that ECM includes "matrix proteins, such as glycoproteins, elastin, and collagen.", see specification at 6, lines 8-9. The term "such as" is open-ended. It does not limit that matrix protein to elastin, and collagen but any glycoproteins. The specification discloses TGF-β at concentration ranging from 0.1 to 100 ng/ml or ascorbic acid at 50 μg/ml stimulates extracellular matrix production as detected by the incorporation of ^{3H}glycine incorporation into the matrix. The production of extracellular matrix depends upon the type of cells and the type of matrix enhancing molecule. For example, the specification discloses acryloyl-PEG tether TGF beta produces less matrix per cell by smooth muscles cells than soluble TGF-beta at 100 ng/ml (see Fig 2, in particular).

The specification does not describe the production of any other matrix protein being produced by any other matrix-enhancing molecule covalently coupling to the scaffold by any cells for the claimed method.

At page 19 of the Brief, the specification has described cells attached to the scaffold. The specification adequately describes cells attached to the scaffold. For example, the specification states that "[p]referred cells for formation of vascular tissue include smooth muscle cells, endothelial cells, and fibroblasts. Preferred cells for formation of connective tissue include

chondrocytes, fibroblasts, and other types of cells that differentiate into bone or cartilage." (Application at 8, 11. 6-9.) Furthermore, the use of various cell types is well known in the art, as the art cited by the Examiner shows. See, e.g., Dinbergs at 29825, col. 1-2 (discussing endothelial cells and smooth muscle cells); '849 Patent, col. 16, Table i (listing a number of cell types); WO 96/27657 at 14, 11.14-27.

Appellants' arguments have been fully considered but are not found to be persuasive. The specification also discloses that the concentration for a specific matrix-enhancing molecule "will depend on the type of cell to be attached to the scaffold.", see specification at 7, lines 15-20. The specification discloses the concentration of the two structurally different Matrix-enhancing molecules, namely TGF-β (protein) and ascorbic acid (chemical). For TGF-β, the optimal concentrations to induce ECM production is in the range of between 1 and 5 ng/ml for *aortic smooth muscle cells* and between 5 and 100 ng TGF- β per ml for *chondrocytes*, which is equivalent to between 4 x 10.6 and 4 x 10^{-3} nmol/ml. However, the optimal concentration for ascorbic acid for chondrocytes and aortic smooth muscle cells was 50 μg/ml, which is a 500 fold more than TGF- β over the same 2-day period (1μg = 1000 ng) for the same cell types, see specification at page 15, Figure 7.

At page 19 bottom of the Brief, the specification generally describes scaffold materials (Application at 5, 11.3-18) and formation of scaffolds (Application at 5, 1.19-p. 6, 1. 5). The specification also provides detailed descriptions of specific scaffolds (Application at 9, 11.23-30; ld at 11, 11.7-22). Furthermore, Applicants' specification states that matrix-enhancing molecules which promote increased production of ECM can be attached to the scaffold material to induce production of matrix proteins.., without substantially increasing cell proliferation. These matrix-enhancing molecules include TGF-13, angiotensin II, insulin-like growth factors and ascorbic acid. (Application at 6, 11. 7-11 .) Applicants' specification also discloses that the concentration for a specific matrix-enhancing molecule "will depend on the type of cell to be attached to the scaffold." (Application at 7, 11.15-16.) In connection, the description provides an example of a specific matrix-enhancing molecule, the concentration needed to elicit ECM production, and the specific type of cell that produces the ECM. (Application at 7, 11. 18-20.) Moreover, Applicants describe other examples of matrix-enhancing molecules (Application at 15, 11.3-14), concentrations (Application at 14, 11. 8-19), and cells (Application at 13, 11. 16-25; ld. at 14, 11.9-14; ld. at 14, 11.21-25) in the Examples section of the Application.

Appellants' arguments have been fully considered but are not found to be persuasive. Claim 24 encompassed any matrix-enhancing molecule at any concentration sufficient to elicit production of extracellular matrix by any cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell for the claimed method.

The specification discloses the scaffold is formed of synthetic or natural polymers, although other materials such as hydroxyapatite, silicone, and other inorganic materials can be used. The scaffold may be biodegradable or non-degradable. There are a number of biocompatible polymers, both degradable and non-degradable. Representative synthetic non-biodegradable polymers include ethylene vinyl acetate and poly(meth)acrylate. Representative biodegradable polymers include polyhydroxyacids such as polylactic acid and polyglycolic acid, polyanhydrides, polyorthoesters, and copolymers thereof. Natural polymers include collagen, hyaluronic acid, and albumin. A preferred material is a hydrogel forming material which is polyethylene glycol-diacrylate polymer, see page 5, lines 3-16. The specification exemplified only polyethylene glycol-diacrylate polymer as the scaffold material attached to TGF-β.

The specification does not describe the production of any other matrix protein being produced by any other matrix-enhancing molecule other than TGF-β covalently tethered to any scaffold other than polyethylene glycol-diacrylate polymer by any cells for the claimed method.

At page 20 of the Brief, appellants argue that throughout the specification and claims, the relationship between the structure of a matrix-enhancing molecule and its function is described. In short, the matrix-enhancing molecule, a class of molecule both well-known in the art and described generically and by example in the specification, is covalently coupled to the scaffold through a tether. This tethered matrix-enhancing molecule retains the function of the untethered matrix-enhancing molecule, to increase the production of extracellular matrix by cells. This structure-function relationship is expressly recited in the claims. For example, Applicants' independent claim 24 itself recites "covalently coupling the matrix-enhancing molecule to the scaffold, wherein the matrix-enhancing molecule is present at a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell." In other words, the matrix-enhancing molecule is present, in a new form (tethered) and amount (effective to produce ECM without increasing proliferation), to perform the function it is known to perform. Likewise, Applicants' specification provides that "[m]atrix-enhancing molecules which promote increased production of

ECM... without substantially increasing cell proliferation." (Application at 6, 11. 7-11.) And Applicants' specification further describes that "[f]or the matrix-enhancing molecules to induce formation of ECM, it is necessary for the molecule to be tethered to the scaffold by a tether." (Application at 7, 11.2-3.) Applicants' specification also describes that "[t]he optimal density [of the matrix-enhancing molecule] will depend on the type of cells to be attached to the scaffold." (Application at 7, 11.15-16.) Additionally, Applicants' specification provides examples of suitable concentrations of specific matrix-enhancing molecules to use with a specific cell type, as well as how a skilled person could determine a particular concentration with reference to TGF-13 and ascorbic acid. (See Application at 7, 11. 16-20; Id. at 13, 1.26- p. 14, 1.7; Id. at 14, 11.15-19; Id. at 14, I. 26-p. 15, 1.2; Id. at 15, 11.9-14.).

Appellants' arguments have been fully considered but are not found to be persuasive. The scope of claim 24 is overly broad. The specification describes the structure of one matrix-enhancing polypeptide, that is, TGF-β covalently coupled to hydrogel made of polyethylene glycol-diacrylate polymer at a concentration between 1 and 5 ng/ml effective to increase matrix production by *aortic smooth muscle cells* and between 5 and 100 ng TGF- β per ml for *chondrocytes* without induce cellular proliferation for a period of two days, see page 5, 7 and 9 of the specification. The specification also discloses ascorbic acid at a concentration of 50 μg/ml to increase matrix production without cellular proliferation by chondrocytes and aortic smooth muscle cells. However, this chemical class of matrix-enhancing molecule was not covalently coupled to hydrogel made of polyethylene glycol-diacrylate polymer, see Materials and Methods, page 10 of specification.

The specification discloses only one matrix enhancing polypeptide TGF-β covalently tethered to one scaffold polyethylene glycol-diacrylate polymer as the scaffold at a concentration between 1 and 5 ng/ml effective to increase matrix production by *aortic smooth muscle cells* and between 5 and 100 ng TGF- β per ml for *chondrocytes* without induce cellular proliferation, one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species of matrix-enhancing molecule to describe the genus for the claimed method. Thus, Applicant was not in possession of the claimed genus. *See University of California v. Eli Lilly and Co. 43 USPQ2d 1398; University of Rochester v. G.D. Searle & Co., 69 USPQ2d 1886 (CA FC2004).*

Applicant is directed to the Final Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

At pages 23-25 of the Brief, appellants argue that that '430 patent fails to anticipate claims 24, 28, 31 and 35 because the concentration of TGF-β1 as taught by the '430 patent is much greater than the amount sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell.

Appellants' arguments with respect to claims 24, 28, 31 and 35 under 35 U.S.C. 102(b) have been considered but are moot in view of the rejection has been withdrawn.

Rejection under 35 U.S.C. 103(a)

Claims 1-2, 4, 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892).

At pages 24-27 of the Brief, the '430 Patent discloses that "biologically active factors to aid in healing or regrowth of normal tissue" may be chemically linked to a collagen-polymer composition. '430 Patent, col. 6, l. 53-col. 7, l. 5. But the '430 Patent does not disclose using biologically active factors at concentrations that elicit production of extracellular matrix without increasing cellular proliferation. Rather, the '430 Patent discloses concentrations sufficient "to stimulate tissue growth to a detectable degree[, and] [t]issue, in this context, includes connective tissue, bone, cartilage, epidermis and dermis, blood, and other tissue." '430 Patent, col. 7, ll. 17-22. Thus, the '430 Patent teaches stimulating cell proliferation and makes no mention of extracellular matrix production. In other words, the '430 Patent teaches proliferation, in contradistinction to the claimed invention. Accordingly, the '430 Patent does not expressly teach the claimed subject matter. Appellants demonstrate on page 24 of the Brief that the concentration of TGFβ exemplified in example 6 of the '430 patent exceeds the claimed amount sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell. There is no basis to combine the '430 Patent and Dinbergs. The '430 Patent teaches away from the claimed invention because it

teaches cell proliferation, the very thing that the claimed invention avoids. The '430 Patent discloses "biologically active factors to aid in healing or regrowth of normal tissue." '430 Patent, col. 6, 1. 53-col. 7, 1.5. And the '430 Patent discloses concentrations sufficient "to stimulate tissue growth to a detectable degree." '430 Patent, col. 7, 11.17-22. Thus, the '430 Patent teaches stimulating cell proliferation and makes no mention of extracellular matrix production, which teaches away from Applicant's claimed invention. The inclusion of the '430 Patent in the obviousness rejections represents clear error that must be reversed. Dinbergs teaches away from the claimed invention because it concerns release of soluble growth factors. Dinbergs at 29827. In particular, Dinbergs compared TGF-β's effect on cell proliferation when released as a bolus or when release was controlled. Dinbergs at 29826. This is in direct conflict with claim 1's requirement to covalently couple the matrix-enhancing molecules to the scaffold, which also necessitates reversal.

Appellants' arguments have been fully considered but are not found to be persuasive.

The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, col. 7, lines 5-10, in particular) covalently coupled to a matrix enhancing molecule such as TGFβ (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular) or insulin like growth factor or combination thereof (see col. 6, line 63, in particular). The reference polyethylene glycol has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The reference tissue engineering is useful for tissue or organ implantation or tissue regeneration (see col. 4, line 28-40, in particular). The '430 patent teaches the concentration of TGFβ range from about 1μg (1μg = 1000 ng) to about 5 mg/ml (see col. 10, lines 63-67, in particular). The '430 patent teaches by tethering factor molecules to the scaffold for implant, the effective amount of the growth factor is substantially reduced (see col. 7, lines 13-10, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired are easily be determined by routine experimentation (see col. 13, lines 11-17, in particular).

However, Dinbergs teach TGF-β at concentration within the claimed range of 1 to 100 ng/ml to inhibit smooth muscle cell proliferation over a two-days time period, see page 29825, col. 1, Figure 3, in particular), see also appellant's argument at page 30 of the Brief.

With respect to argument that the '430 patent teaches cell proliferation, it is noted that at the concentration taught by Dinbergs does not increase smooth muscle cell proliferation over the same 2-days time period as disclosed in the instant specification, see reference FIG3A-B, and also see instant specification at page 14, lines 15-19 and Figure 4.

With respect to the argument that there are no mentioned of eliciting extracellular matrix production, it is well known to one of ordinary skill in the art at the time the invention was made that "TGFbeta is known to increase production of extracellular matrix proteins such as collagen by vascular smooth muscle cells (SMC), see instant specification at page 6, lines 12-25. It is because of this property of TGFbeta, TGF is widely used and incorporated into biomaterials for tissue engineering and wound healing, see references cited in the specification, and references of record cited by the Examiner, see '849 patent, col. 12, lines 57-67 bridging col. 13, lines 1-2.

With respect to the argument that Dinbergs teaches release of soluble growth factors, the rejection is based on the combined teachings of the '430 patent and the Dinbergs et al. The '430 patent teaches tethering factor molecules to the scaffold for implant so that the effective amount of the growth factor is substantially reduced (see col. 7, lines 13-10, in particular). The test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference and not is it that the claimed invention must be expressly suggested in any one or all of the references; but rather the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. In re Keller, 642 F.2d 413, 208 USPQ 871 (CCPA 1981). See MPEP 2145.

At page 27 of the Brief, appellants argue that there is no reasonable expectable of success in combining the '430 patent and Dinbergs to achieve the claimed invention because Dinbergs teaches sustained release of TGFβ does a better job of inhibiting proliferation than promoting it.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellants' assertion that Dinbergs et al teach away the claimed invention, Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The concentration 1-10 ng/ml taught by Dinbergs does not increase smooth muscle cell proliferation over the same 2-days time period as disclosed in the instant specification, see specification at page 14, lines 15-19 and Figure 4. Further, the '430 patent teaches the growth factor may be attached a polymer tether such as PEG

before attached to the matrix such as collagen. By tethering factors to the implant, the effective amount of factor is *substantially reduced* (see col. 7, lines 5-10, in particular).

At page 28 of the Brief, appellants argue that Dinbergs never studied extracellular matrix production. Rather, Dinbergs studied how growth factors interact with the extracellular matrix, and how growth factors released from the extracellular matrix affect cell proliferation. In fact, for Dinbergs's studies, the cells were removed from their extracellular matrix before adding any growth factors. See Dinbergs at 29823 (explaining in the fight hand column under the subheading "Extracellular Matrix Incorporation and Release of Growth Factors" that "... to remove the solubilized cells, leaving extracellular matrix coating the bottom of the wells."). Dinbergs simply does not study using growth factors to increase extracellular matrix production while minimizing cell proliferation. Furthermore, Dinbergs's Figures 3A and 3B make no mention of eliciting extracellular matrix formation at all. What is mentioned is that TGF-β can inhibit the proliferation of certain cells. But with reference to smooth muscle cells, Dinbergs actually demonstrates cell proliferation. This is clearly seen from Figure 3B, in which the cell number increased after a single day. And by the second day of the experiment, about an additional 25,000 cells have proliferated.

Appellants' arguments have been fully considered but are not found to be persuasive.

Dinbergs *et al* teach a method for making a tissue engineering scaffold such as alginate/heparin-sepharose microsphere for inducing formation of extracellular matrix by cells such as endothelial cells and smooth muscle cells bound to said scaffold comprising coupling various matrix-enhancing molecule such as bFGF or TGFβ in a concentration 1-10 ng/ml (See Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). The reference TGFβ is effective to elicit production of extracellular matrix (see page 29822, column 2, last paragraph, in particular) without increasing cellular proliferation (See Fig 2B, Fig 3B, Abstract, in particular). Dinbergs *et al* teach TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). In contrast to appellants' assertion that

Dinbergs teach cell proliferation of certain cell, Dinbergs's teach TGF-β at the concentration used by the instant disclosure can inhibit the proliferation of both smooth muscle cells (Fig. 3B) and endothelial cells (Fig. 3A), especially at a two-days time period. This is consistent with the same time frame as disclosed in the specification, see page 14, lines 15-19 and FIG 4 of instant application.

With respect to the argument that Dinbergs et al does not teach eliciting extracellular matrix formation, given the concentration as taught by Dinbergs is within the claimed range of the claimed method, Dinbergs et al teach TGF-β inhibits cell proliferation of SMC and endothelial cells over a two-days time period (see FIG 3A-B) and TGF-β obviously has the same effect of inducing extracellular matrix production. Production of extacellular matrix is an inherent property of TGF-β. As evidenced by the disclosure of instant specification, "TGF-β is known to increase production of extracellular matrix proteins by vascular SMCs growing in culture (Amento, et al. Arterioscler. Thromb. 11:1223-1230 (1991); Lawrence, et al., J. Biol. Chem. 269:9603-9609 (1994); Plenz, et al., Atherosclerosis 144:25-32 (1999)). TGF-β, through production by SMCs naturally during vessel injury or by gene transfer, can also increase ECM production by SMCs in vivo (Majesky, et al., J. Clin. Invest. 88:904-910 (1991); Nabel, et al., Proc. Natl. Acad. Sci. USA 90:10759-10763 (1993)). Cultured fibroblasts have also been shown to increase collagen synthesis (Clark, et al., J. Cell Sci. 108:1251-1261 (1995); Eickelberg, et al., Am. J. Physiol. 276:LS14-L824 (1999)) and proteoglycan synthesis (Heimer, et al., J. Mol. Cell Cardiol. 27:2191-2198 (1995)) in the presence of TGF-β. Further, topical delivery of TGF-β (Puolakkainen, et al., J. Surg. Res. 58:321-329 (1995)) and delivery to TGF-β through a collagen scaffold (Pandit, et al., J. Invest. Surg. 12:89-100 (1999)) have been shown to enhance wound healing.", see page 6 of the specification.

At page 29 of the Brief, appellants argue that Dinbergs does not teach coupling. The growth factors in Dinbergs are not coupled to a polymeric scaffold, but instead are encapsulated by a polymeric matrix and released as soluble growth factors. Furthermore, the TGF-β used in Figures 3A and 3B of Dinbergs was "solvent-cast within EVAc microspheres," rather than tethered to a scaffold. See Dinbergs at 29824, col. 1, 5th full paragraph. The growth factors in Dinbergs are not coupled to a polymeric scaffold, but instead are encapsulated by a polymer and released as soluble growth factors, quite unlike the invention of independent claim 1.

Appellants' arguments have been fully considered but are not found to be persuasive. This rejection is based on the teachings of the combined teachings of the '403 patent and Dinbergs et al. The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The reference polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). In fact, the '430 patent teaches factors may be attached to the free polymer end by the same method used to attached PEG to collagen matrix. The advantage of tethering factor molecules to the implant, the effective amount of factor is substantially reduced (see col. 7, lines 5-10, in particular). Further, the reference polymeric scaffold collagen or alginate are the scaffold as defined by the specification, see page 5, "the scaffold is formed of synthetic or natural polymers, although other materials such as hydroxyapatite, silicone, and other inorganic materials can be used. The scaffold may be biodegradable or non-degradable. There are a number of biocompatible polymers, both degradable and non-degradable. Representative synthetic non-biodegradable polymers include ethylene vinyl acetate and poly(meth)acrylate. Representative biodegradable polymers include polyhydroxyacids such as polylactic acid and polyglycolic acid, polyanhydrides, polyorthoesters, and copolymers thereof. Natural polymers include collagen, hyaluronic acid, and albumin. A preferred material is a hydrogel. A particularly preferred hydrogel forming material is a polyethylene glycol-diacrylate polymer, which is photopolymerized. Other hydrogel materials include calcium alginate and certain other polymers that can form ionic hydrogels that are malleable and can be used to encapsulate cells."

At paragraph bridging pages 29-30 of the Brief, appellants argue that Dinbergs does not teach the claimed concentration. At the outset, the 1-10 ng/ml figure cited by the Examiner has nothing to do with Dinbergs' microsphere studies. This is the growth factor concentration added to cell-free extracellular matrix for the ECM incorporation and release experiment shown in Figure 5. See Dinbergs at 29823, col. 2, fourth full paragraph-29824 ("Extracellular Matrix Incorporation and Release of Growth Factors"); Id. at 29825, col. 2, third fill paragraph-29826 ("bFGF and TGF-β Release from the Extracellular Matrix"). Dinbergs specifically discloses

forming EVAc-BSA-TGF-131 microspheres by adding TGF-β at a concentration of 3 ng TGF-β per microsphere. See Dinbergs at 29823, col. 2, third full paragraph. And each microsphere releases 0.4 ng TGF-J3. See 29825, col. 1, second full paragraph ("one EVAc-BSA-TGF-J31 microsphere releases 0.4 ng TGF-β"); see also Dinbergs at 29823, col. 1, fifth full paragraph-29823, col. 2 ("Cell Proliferation Assay"). Even if we incorrectly assume a single microsphere could be a tissue engineering scaffold, the microsphere falls short of the claimed concentration.

Appellants' arguments have been fully considered but are not found to be persuasive. Given that three ng TGF-β per microsphere, 5-10 spheres were placed in 1 ml of PBS is equivalent to 15-30 ng/ml. Each microsphere released 0.4 ng TGF-β, the minimum TGF-β released by 5-10 microsphere is in the range of 2 to 4 ng/ml, which is still within the claimed range of 1-100 ng/ml. In contrast to appellants' argument Dinbergs does not teach the use of more than one spheres, page 29823, col. 1, last paragraph teaches the use of ethylene-vinyl acetate copolymer (EVAc)-bovine serum albumin (BSA)-TGF-β controlled release microspheres with and without TGF-β1 were placed into the culture wells.

With respect to the argument that a single microsphere would not, by itself, be surgically implanted in the body of a mammal quite unlike the scaffolds of the present invention, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. In re Keller , 642 F.2d 413, 208 USPQ 871 (CCPA 1981); In re Merck & Co., Inc. , 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). See MPEP 2145. The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The scaffold as taught by the '403 patent has the same use as appellants' scaffold.

At page 32 of the Brief, appellants argue that the prior art did disclose the use of collagen, polyethylene glycol polymers, hydron, and other hydrogels to deliver growth factors, but each one of these materials was found to be problematic, e.g., demonstrating "shorter or suboptimal release times and difficulty in handling." See id. none of these disclosures cited in Dinbergs, nor even Dinbergs itself, disclosed tethering a growth factor to a scaffold, much less

covalent tethering. Nowhere in the above excerpt is "covalent", "tethering", or a "scaffold" mentioned. See id. the embodiment cited by the Examiner involving the use of alginate to release TGF-β actually discloses the "complete" and "rapid" release of TGF-β during a 2 hour period. The excerpt explicitly teaches away from the sustained release of growth factors, which constitutes a teaching away from Appellants' invention.

Appellants' arguments have been fully considered but are not found to be persuasive. Again, the rejection is based on the combined teachings of the references. Claim 1 does not recite the scaffold is from a specific polymer such as collagen, polyethylene glycol polymers, hydron, and other hydrogels. Only claim 8 recites the scaffold is a polymer selected from the group consisting of alginate, collagen, hyaluronic acid, and polyethylene glycol polymer. .The '430 patent teaches tethering factor molecules to the scaffold for implant so that the effective amount of the growth factor is substantially reduced (see col. 7, lines 13-10, in particular).

Dinbergs et al teach ethylene-vinyl acetate copolymer (EVAc) as the polymer tethered to -bovine serum albumin (BSA)-TGF-β, see page 29823, col. 1, last paragraph, in particular). Dinbergs et al teach other matrix such as alginate/heparin-sepharose (see page 29823, col. 2, in particular). Further, the '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFβ (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular) or insulin like growth factor or combination thereof (see col. 6, line 63, in particular). The reference polyethylene glycol has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The reference tissue engineering is useful for tissue or organ implantation or tissue regeneration (see col. 4, line 28-40, in particular).

With respect to tethering, the '430 patent teaches growth factors such as TGF-β may be attached to the free polymer end by the same method used to attached PEG to collagen matrix. The advantage of tethering factor molecules to the implant, the effective amount of factor is substantially reduced (see col. 7, lines 5-10, in particular).

At paragraph bridging pages 32-33 of the Brief, the combination of the '430 Patent and Dinbergs is not only improper, but moreover, it fails to obviate claim 1. Neither reference discloses the benefit of enhancing extracellular matrix formation without increasing cellular

proliferation, and neither discloses coupling TGFβ to a polymeric scaffold in an effective density between 1-100 ng/mL. The '430 Patent does not disclose a method of enhancing production of extracellular matrix molecules. Nor does the '430 Patent disclose TGFβ in effective density of 1-100 ng TGFβ/ml.

Appellants' arguments have been fully considered but are not found to be persuasive. The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The reference polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). In fact, the '430 patent teaches factors may be attached to the free polymer end by the same method used to attached PEG to collagen matrix. The advantage of tethering factor molecules to the implant is the effective amount of factor required is substantially reduced (see col. 7, lines 5-10, in particular). The density or concentration of TGFβ ranges from about 1μg/ml to about 5mg/ml (see col. 10, lines 65-67, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired is within the purview of one ordinary skill in the art, these parameters may easily be determined by routine experimentation (see col. 13, lines 9-17, in particular). Although the '430 patent does not explicitly teach the claimed range of between 1 and 100 ng, Dinbergs et al teach TGFβ at concentration as low as 0.4 ng/ml/well could still inhibit smooth muscle cell proliferation and endothelial cell proliferation over a 2-days time period. The same time period as shown in the instant specification. Whether the claimed invention really inhibits cellular proliferation of smooth muscle cells and endothelial cells over a longer period as shown by Dinbergs et al is a matter of debate since the specification discloses only cell proliferation of smooth muscle cell over a 2-day time period, see page 14 of specification. With respect to production of extracellular matrix, TGFβ is known to induce the production of extracellular matrix, see page 6 of the specification. The specification also discloses in the presence of 0.04pmol/ml of TGF, more hydroxyproline and thus more collagen, was produced by smooth muscle cells grown in the presence of *either* soluble or tethered TGF-β than when no TGF-β was present (see page 13, line 26-9, in particular).

Finally, obviousness does not require absolute predictability but only the reasonable expectation of success. See In re Merck and Company Inc., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986); and In re O'Farrell, 7 USPQ2d 1673 (Fed. Cir. 1988).

Claims 5, 7 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892) as applied to claims 1-2, 4, 8 and 9 and further in view of US Pat No. 5,935,849 (Aug 10, 1999; PTO 892).

At page 33-34 of the Brief, appellants argue that not only is the combination of the '430 Patent, Dinbergs, and the '849 Patent improper, it also fails to obviate claims 7 and 8. (See Final Office Action at 12.) As demonstrated above, the '430 Patent and Dinbergs do not obviate Applicants' independent claim 1. The '849 Patent does not teach the subject matter missing from the '430 Patent and Dinbergs. As claim 1 is nonobvious over the prior art for the reasons described above, dependent claims 7 and 8 are similarly nonobvious because they include the limitations of their respective base claim, which Applicants have shown above to be allowable. The '849 Patent concerns bioartificial organs (BAOs). BAOs are devices that encapsulate cells within a semi-permeable membrane. '849 Patent, col. 5, 11.9-40. According to the '849 Patent, the cells encapsulated by the BAO may grow on the inner luminal surface of the membrane, or they may grow on an inner surface encapsulated within the BAO. '849 Patent, col. 16, 11.43-46. But the '849 Patent does not disclose tethers, matrix-enhancing molecules covalently coupled, or suitable concentrations of TGF-β.

Appellants' arguments have been fully considered but are not found to be persuasive.

The combined teachings of the '430 patent and Dinbergs et al have been discussed supra.

The claimed invention in claim 5 differs from the teachings of the references only in that the method wherein the matrix-enhancing molecule is ascorbic acid.

The claimed invention in claim 7 differs from the teachings of the references only in that the method wherein the scaffold is a hydrogel.

The claimed invention in claim 8 differs from the references only in that the method wherein the scaffold hydrogel is alginate and combination thereof.

The '849 patent teaches a method of making a tissue engineering scaffold such as bioartificial organ (BAO) using scaffold such as hydrogel or alginate or collagen (see col. 17,

lines 66 through col. 18, col. 19, lines 22, col. 20, lines 42-36, summary of invention, in particular) where the luminal surface is tether to a polymer such as poly-d-lysine (see col. 18, line 30-35, in particular) and then coupling to matrix enhancing molecule such as RGD containing sequence (see col. 18, lines 36-51, in particular) or TGF beta and/or ascorbic acid (see col. 12, line 56-67, in particular). The '849 patent teaches TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells while ascorbic acid and TGFbeta1 increase extracellular matrix such as collagen biosynthesis (see col. 12, lines 57-67, Table 1, in particular). The reference method further comprises providing cells such as fibroblast or endothelial cells attached within the tissue-engineering scaffold (see col. 16, Table 1, Col. 19, line 29, in particular). The reference method is useful for implantation and controlling distribution of cells within the bioartificial organ (see claims of '849 patent).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the collagen in the tissue engineering scaffold comprising collagen covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix enhancing molecule such as TGFβ as taught by the '430 patent for the hydrogel such as alginate as taught by the '849 patent for a method of making a tissue engineering scaffold comprising the hydrogel such as alginate covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix-enhancing molecule such as TGFβ and/or ascorbic acid as taught by the '430 patent, Dinbergs et al and the '849 patent. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because TGF beta is useful for inducing differentiation of fibroblast cells, and also as a growth inhibitor of keratinocytes and endothelial cells, while ascorbic acid and TGFbeta1 increase collagen biosynthesis as taught by the '849 patent (see col. 12, lines 57-67, Table 1, in particular). The use of engineering scaffold is useful to control cell number, cell distribution and attachment in organ transplant as taught by the '849 patent. Dinbergs *et al* teach TGFβ is useful for inhibiting cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular) and the advantage of tethering growth factor via a polymer to the matrix is that less effective amount of growth is required (see col. 7, lines 5-10, in particular).

With respect to argument that the '849 Patent does not disclose tethers, matrix-enhancing molecules covalently coupled, or suitable concentrations of TGF-β, the '849 patent teaches a method of making a tissue engineering scaffold such as bioartificial organ (BAO) using scaffold such as hydrogel or alginate or collagen (see col. 19, lines 22, col. 20, lines 42-36, summary of invention, in particular) covalently coupling to an inner matrix by a tether such as poly-d-lysine (see col. 18, line 30-35, in particular) coupling to matrix enhancing molecule such as RGD containing sequence (see col. 18, lines 36-51, in particular) or TGF beta and/or ascorbic acid (see col. 12, line 56-67, in particular). Even if the '849 patent does not discloses tethers as agued (but it does), the '430 patent teaches growth factors such as TGF-β may be attached to the free polymer end by the same method used to attached PEG to collagen matrix. The advantage of tethering factor molecules to the implant is the effective amount of factor required is substantially reduced (see col. 7, lines 5-10, in particular).

At paragraph bridging pages 35 of the Brief, Appellants argue that the '849 Patent fails to disclose TGF-β "in a density between 1 and 100 ng TGF-13/mL or in a concentration of between about 4×10^{-6} and 4×10^{-3} nmol/ml," as recited by claim 1. In fact, the' 849 Patent makes no mention of stimulating ECM production by cells associated with a tissue engineering scaffold. See '849 Patent col. 14, I. 23-col. 15, 1.67 (describing the use of ECM molecules to control cell growth and differentiation). And although the '849 Patent does describe a number of matrix-enhancing molecules, it does not disclose any concentration suitable for the claimed invention. See '849 Patent, col. 11, 1.54- col. 14, 1. 6. In fact, portions of the '849 Patent are specifically directed to promoting cellular proliferation, which teaches away from the claimed invention. See '849 Patent col. 20, 11.42-50 ("The core can comprise a liquid medium sufficient to maintain cell growth.").

Appellants' arguments have been fully considered but are not found to be persuasive. This rejection is based on the teachings of the combined teachings of the '403 patent, Dinbergs et al and the '849 patent. Dinbergs et al teach TGFβ growth factor concentration added to cell-free extracellular matrix for the ECM incorporation and release experiment shown in Figure 5. See Dinbergs at 29823, col. 2, fourth full paragraph-29824 ("Extracellular Matrix Incorporation and Release of Growth Factors"); Id. at 29825, col. 2, third fill paragraph-29826 ("bFGF and TGF-β Release from the Extracellular Matrix"). Dinbergs specifically discloses forming EVAc-BSA-TGF-131 microspheres by adding TGF-β at a concentration of 3 ng TGF-β per microsphere. See

Dinbergs at 29823, col. 2, third full paragraph. And each microsphere releases 0.4 ng TGFβ. See 29825, col. 1, second full paragraph ("one EVAc-BSA-TGF-J31 microsphere releases 0.4 ng TGF-β"); see also Dinbergs at 29823, col. 1, fifth full paragraph-29823, col. 2 ("Cell Proliferation Assay"). Given that three ng TGF-β per microsphere, 5-10 spheres were placed in 1 ml of PBS is equivalent to 15-30 ng/ml. Each microsphere released 0.4 ng TGF-β, the minimum TGF-β released by 5-10 microsphere is in the range of 2 to 4 ng/ml, which is still within the claimed range of 1-100 ng/ml. Further, Dinbergs teach the use of more than one spheres, page 29823, col. 1, last paragraph teaches the use of ethylene-vinyl acetate copolymer (EVAc)-bovine serum albumin (BSA)-TGF-β controlled release microspheres with and without TGF-β1 were placed into the culture wells.

In response to appellants' argument that portions of the '849 Patent are specifically directed to promoting cellular proliferation, which teaches away from the claimed invention, the '849 patent teaches TGF-β1 may be used to arrest or inhibit cell proliferation or to induce cell differentiation and induces extracellular matrix synthesis such as collagen biosynthesis (see col. 12, lines 57-67, bridging col. 13 line 1-2, in particular).

At paragraph bridging pages 35-36 of the Brief, appellants argue that the '849 Patent teaches using unbound chemical compounds or growth factors that inhibit cell proliferation or induce differentiation to control cell distribution within a BAO. As shown above, the '430 Patent teaches away from the claimed invention because it concerns cell proliferation, the very thing that the claimed invention avoids; and Dinbergs teaches away from the claimed invention because it concerns release of soluble growth factors, which is in direct conflict with claim 1's requirement to covalently couple TGF-β to the scaffold. Because they teach different outcomes, the '430 Patent, Dinbergs, and '849 Patent are incompatible and cannot be combined. The inclusion of the '849 Patent in the obviousness rejections represents clear error that must be reversed. The teachings of the '430 Patent and Dinbergs are described above. (See supra Section V.) The '849 Patent teaches that proliferation-inhibiting or differentiation-inducing compounds, like TGF-β, may used to control cell distribution within a BAO. '849 Patent col. 12, 11.46-51. In connection, the '849 Patent teaches exposing cells to proliferation-inhibiting compounds, like TGF-β '849 Patent col. 12, 11.57-62. The Examiner contends that "ascorbic acid and TGFbeta increase collagen biosynthesis as taught by the '849 patent (see col. 12, line 57-67, Table 1, in particular)." (Final Office Action at 13.) Table 1 however, describes how ECM molecules, growth factors, and

chemical compounds influence proliferation or differentiation of various cell types. There is nothing in Table I, or the text of the '849 Patent, that teaches increased collagen biosynthesis by ascorbic acid and TGF-β. For ascorbic acid and TGF-β, what is disclosed is that together these promote the proliferation of neuroendocrine cells and inhibit BHK cells. See '849 Patent col. 16, Table 1. The '849 Patent makes no reference to increased collagen biosynthesis.
In light of these teachings, a combination of the '430 Patent, Dinbergs, and the "849 Patent would change the basic principle under which each was designed to operate. The '430 Patent was designed for sustained release of growth factors, such as TGF-β to "stimulate tissue growth to a detectable degree." '430 Patent, col. 7, 11. 18-22; col. 19, 11. 10-56 and Figure 2. But according to the '849 Patent and Dinbergs, exposure to TGF-β would actually inhibit cellular proliferation, preventing tissue growth to a detectable degree. Therefore, there would be no motivation to combine the teachings of the '430 Patent, Dinbergs, and the '849 Patent.

Appellants' arguments have been fully considered but are not found to be persuasive. The '849 patent teaches TGF-β1 may be used to arrest or inhibit cell proliferation or to induce cell differentiation and induces extracellular matrix synthesis such as collagen biosynthesis (see col. 12, lines 57-67, bridging col. 13 line 1-2, in particular). The '849 patent teaches TGFβ at concentration of 2.5 ng/ml and ascorbate (100μM) reduced mitosis (cell proliferation), see col. 24, lines 40-49, in particular).

In contrast to appellant's argument that there would be no motivation to combine the teachings of the '430 Patent, Dinbergs, and the '849 Patent, the combined teachings particularly the '430 provide clear direction, motivation and expectation of success in making a tissue engineering scaffold by covalently coupling to TGF-β to collagen matrix via a PEG polymer tether at a concentration or density such as 2.5 ng/ml as taught by the '849 patent or as low as to 0.4 ng to 4 ng/ml as taught by Dinbergs to inhibits cell proliferation while inducing matrix formation by cell attached to the matrix as taught by the '849 patent. The advantage of tethering factor molecules to the implant is the effective amount of factor required is substantially reduced as taught by the '430 patent (see col. 7, lines 5-10, in particular). The '849 patent teaches the use of TGF-β1 and ascorbic to inhibit cell proliferation while increase collagen synthesis is known in the art as early as 1992, see col. 12, lines 57 through col. 13, lines 1-2. The '849 patent teaches control of cell distribution in a tissue engineering scaffold such as BAO may be achieved by providing chemical compound or growth factor which inhibits cell proliferation of induce cell

differentiation (see col. 12, lines 51-51, in particular). Dinbergs teach tether TGF-β at concentration as low as 0.4 ng inhibit smooth muscle cell proliferation at a 2-days time period.

At paragraph page 37 of the Brief, appellants argue that even if the '430 Patent, Dinbergs, and the '849 Patent could properly be combined, such a combination would fail to obviate claim 1. None of the references alone or in combination discloses enhancing extracellular matrix formation without increasing cellular proliferation, or the benefit of doing so. Indeed, at an even more basic level neither reference teaches or suggests "covalently coupling the matrix-enhancing molecules to the scaffold in an effective density to elicit production of extracellular matrix without increasing cellular proliferation," as recited in independent claim 1. As discussed above in Section V.C, the '430 Patent and Dinbergs have not been shown to teach this limitation. Nor has the '849 Patent been shown to supply this missing limitation. Nowhere has the '849 Patent been shown to discuss suitable concentrations of matrix-enhancing molecules, much less "an effective density to elicit production of extracellular matrix without increasing cellular proliferation." Accordingly, even if the cited references are combined, the Examiner has failed to show how the combination obviates claim 1. As claim 1 is nonobvious over the prior art, dependent claims 7 and 8 are similarly nonobvious.

Appellants' arguments have been fully considered but are not found to be persuasive. In contrast to appellant's assertion that none of the references alone or in combination discloses enhancing extracellular matrix formation without increasing cellular proliferation, the '849 patent teaches TGF-β1 may be used to arrest or inhibit cell proliferation or to induce cell differentiation and induces extracellular matrix synthesis such as collagen biosynthesis (see col. 12, lines 57-67, bridging col. 13 line 1-2, in particular). The '849 patent teaches TGFβ at concentration of 2.5 ng/ml and ascorbate (100μM) reduced mitosis (cell proliferation), see col. 24, lines 40-49, in particular).

In contrast to appellants' assertion that no reference teaches covalently coupling the matrix-enhancing molecules to the scaffold, the '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19,

in particular). The reference polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The advantage of covalently coupled to a matrix enhancing molecule (growth factor) to the scaffold is that substantially less effective amount of growth factor is required, see '430, col. 7, lines 5-10, in particular).

Claim 8 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892) as applied to claims 1-2, 4, 8 and 9 and further in view of WO 94/23740 (of record, Oct 1994, PTO 1449) or WO 96/27657 (of record, Sept 1996; PTO 1449).

At pages 37-39 of the Brief, appellants' argument that WO 94/23740 and WO 96/27657 do not teach the subject matter missing from the '430 Patent and Dinbergs. Moreover, the proposed combination of the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 is improper because the Examiner has not provided a sufficient teaching, suggestion, or motivation in the prior art to make such a combination, as discussed herein. Such improper combination cannot properly be used to obviate the subject claims. WO 94/23740 fails to disclose a method of making a tissue engineering scaffold, or that matrix enhancing molecules may be coupled to such a tissue engineering scaffold. The Examiner contends that WO 94/23740 teaches methods of making a tissue engineering scaffold. The WO 94/23740 simply teaches modifying a growth factor, like TGF-β, by covalently binding a water soluble hydrophilic polymer, like PEG. WO 94/23740 at 5, 11.8-11, 5, 1.35-6, 1. 2. As taught in WO 94/23740, the polymer (e.g., PEG) is not a scaffold. Rather, the polymer is a substance that is conjugated with the growth factor, whereupon the polymer-conjugated growth factor is administered to an animal in solution. See, e.g., WO 94/23740 at 12, 11.28-33. As taught in WO 94/23740, the polymers disclosed in WO 94/23740 are not themselves scaffolds, nor does WO 94/23740 even disclose tethering its polymer-conjugated growth factors to a scaffold. As taught in WO 94/23740, the polymers disclosed in WO 94/23740 are systemically administered, whereas the scaffolds of the present invention are locally administered. WO 94/23740 at 16, 11.24-32. Accordingly, WO 94/23740 does not disclose methods of making a tissue engineering scaffold, much less methods of making

a tissue engineering scaffold involving covalently coupling a matrix enhancing molecule like TGF-~ to the scaffold, a limitation that is recited in Applicants' claim 1.

Appellants' arguments have been fully considered but are not found to be persuasive.

The combined teachings of the '430 patent and Dinbergs et al have been discussed supra.

The claimed invention in claim 8 differs from the combined teachings of the references only in that the method wherein the scaffold is a hyaluronic acid or polyethylene glycol polymer instead of collagen.

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 covalently coupling to polyethylene glycol (See page 12, line 11, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication teaches the method of making a tissue engineering scaffold comprising coupling TGFβ to a polymer is useful for stimulation of bone formation at a lower dose (See abstract, in particular).

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) covalently coupled to a scaffold such as hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate, (See page 17, line 8, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the scaffold collagen in the tissue engineering scaffold comprising collagen covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol and matrix enhancing molecule such as TGFβ as taught by the '430 patent for the polyethylene glycol as taught by the WO 94/23740 publication or the hyaluronic acid as taught by the WO 96/27657 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because polyethylene glycol covalently to TGFβ2 is useful for stimulation of bone formation at a lower dose as taught by the WO 94/23740 publication (See abstract, in particular). The WO 96/27657

publication teaches hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate coupled to TGFβ is useful for localized the desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular).

With respect to the argument that bone formation taught by the WO 94/23740 is not a scaffold, the WO 94/23740 publication teaches a method of inhibiting cell proliferation, see reference page 2, lines 14-15, stimulates bone formation, see reference page 4, lines 5-6, by *stimulates matrix production* such as bone deposition, see page 6, lines 13-15.

With respect to the argument that the polymers disclosed in WO 94/23740 are systemically administered, whereas the scaffolds of the present invention are locally administered, the 'WO 94/23740 teaches the reference growth factor TGF-β is tethered to hydrophilic polymer PEG via crosslinker hydroxysuccinimide or dicarboxylic acid (see page 10-11, in particular). The reference conjugate can be surgically implanted subdermally or within the peritoneal cavity (see page 15, lines 1-3, in particular). Further, instant specification discloses the scaffold is formed of synthetic or natural polymers, although other materials such as hydroxyapatite, silicone, and other inorganic materials can be used. The scaffold may be biodegradable or non-degradable. There are a number of biocompatible polymers, both degradable and non-degradable. Representative synthetic non-biodegradable polymers include ethylene vinyl acetate and poly(meth)acrylate. Representative biodegradable polymers include polyhydroxyacids such as *polylactic acid* and polyglycolic acid, polyanhydrides, polyorthoesters, and copolymers thereof, see page 5, line 3-13. The WO 94/23740 publication teaches "hydrophilic polymer" is a synthetic or natural polymer such as PPG, PEG, POE, polytrimethylene glycols, *polylactic acid*, derivative thereof, polyethylene glycol, polysaccharide, see page 5, lines 8-35.

At page 39 to 40 of the Brief, appellants argue that WO 96/27657 does not disclose covalently coupling a matrix enhancing molecule to a scaffold to increase extracellular matrix production without increasing cellular proliferation. According to WO 96/27657, various growth factors are covalently attached to a scaffold via a polymer tether. See WO 96/27657 at 10, 1.20-11, 1.10, 10, 11.3-5; 6, 11. 13-14. The growth factors of WO 96/27657, however, are used at concentrations that stimulate cellular proliferation. See WO 96/27657 abstract & claims 1, 12, 31. Accordingly, the Examiner has failed to show that WO 94/23740 discloses covalently coupling a

matrix enhancing molecule to a scaffold to increase extracellular matrix production without increasing cellular proliferation, as recited in Applicants' claim 1.

Appellants' arguments have been fully considered but are not found to be persuasive. The rejection is based on the combined teachings of the '430 patent, Dinbergs *et al* and WO 94/23740 or WO 96/27657.

The WO 96/27657 publication teaches biocompatible polymers and spacer molecules are known in the art and most are expected to be suitable for forming tethers. The only important characteristics are biocompatibility and flexibility, see page 8, lines 23-28. The WO96/27657 publication teaches various scaffolds, i.e., none biodegradable such as polystyerene, polyethylene vinyl acetates, polypropylenes, polymethacyrlates, polyacrylates, polyethylenes, polyethylene oxides, glass, polysilicates, etc while biodegradable scaffold such as polyanhydrides, polyglycolic acid, polyhydroxy acids such as polylactic acid, polyglycolic acid copolymer, collagen, alginate, hydrosyapatite (see page 9, lines 8-29, in particular), hyaluronic acid, starch, alginate (see page 17, lines 1-12, in particular) and can be any shape such as fibers, woven fibers, shaped polymers, particles, microparticles, sheets, sponge, or membrane (see page 9, line 25-29, page 10, lines 1-6, page 12, page 17 of WO 96/27657, claims 1-13 of WO 96/27657, in particular). The WO 96/27657 publication also teaches photocrosslinkable agents (see page 12, lines 1-12, in particular). The WO 96/27657 publication teaches immobilization of tissue factor (tethered tissue factor) prevents the factor from diffusing away from the site and consequently allows a much more highly targeted form of delivery than over other methods. Besides this concentration effect, tethering has other powerful advantages, it prevents receptor internalization upon ligand binding and considerably less growth factor is required because cells do not internalize and degrade the growth factor, see page 5, lines 13-30, in particular). The WO 96/27657 publication concludes immobilized growth factor on flexible tethers allow the ligand receptor complex to aggregate in the cell membrane (see page 6, lines 6-8, abstract, in particular).

At page 40 of the Brief, appellants argue that the '430 Patent concerns cell proliferation, and emphasizes "healing or regrowth of normal tissue." See, e.g., '430 Patent, col. 6, 1. 53-col. 7, 1.5. Similarly, WO 94/23740 and WO 96/27657 emphasize cell growth and proliferation. See, e.g., WO 94/23740 at p. 4, 11.1-19, p. 20, 11. 8-12 ("Very highly significant proliferation of osteoblast-like cells was observed in the femur slides of mice treated at 3 μg with rTGF-β2 (PEG 5000)6 OR rTGF-β2 (PEG 5000)4 or rTGF-β2 (PEG 35,000)1-3, as compared with controls.");

WO 96/27657 at p.3, 11. 21-22 ("It is therefore an object of the invention to provide a cell and tissue growth substrate that stimulates long-term target cell growth."). In contrast, the present invention discloses, among other things, increasing production of extracellular matrix while minimizing cell growth. Accordingly, one of ordinary skill attempting to increase production of extracellular matrix without increasing cell growth would not be motivated to combine the teachings of the '430 Patent and WO 94/23740 or WO 96/27657, because these references emphasizes increasing cell growth, in direct contrast to the present invention.

Appellants' arguments have been fully considered but are not found to be persuasive. With respect to the argument that the WO 94/23740 teaches emphasize cell growth and proliferation rather than inhibition of cell proliferation, the WO 94/23740 publication teaches a method of inhibiting cell proliferation, see reference page 2, lines 14-15, stimulates bone formation, see reference page 4, lines 5-6, by stimulates matrix production such as bone deposition, see page 6, lines 13-15. The WO 96/27657 teaches the use of cell growth substrates with tethered cell growth factor molecule to stimulate tissue replacement by limiting the amount of tissue growth and eliminating the need to remove the tissue scaffold once implanted (see page 9, lines 5-7, page 10, lines 3-10, in particular). The WO 96/27657 teaches depending on the type of cells, cells have a complex, nonlinear response to the concentration of factor (see page 2, lines 12-27, in particular) and the mitogenic effects observed for the target cell in vitro may not correlate with tissue growth factor *in vivo* due to internalization of tissue factor factors, loss of responsiveness to growth factors and the amount of time cell growth must be stimulated for the particular application (see page 2, lines 20-29, page 17, lines 13 through page 18, lines 1-15. Further, this rejection is based on a combination of references.

At page 41 of the Brief, Appellant argue that Dinbergs teaches that TGF-β weakly inhibits cellular proliferation, which contrasts with the intent of WO 94/23740 and WO 96/27657, which is to promote cellular proliferation. Because Dinbergs teaches the inhibition of cell growth, while WO 94/23740 and WO 96/27657 teach the promotion of cell growth, the references cannot be combined. One of ordinary skill attempting to increase production of extracellular matrix without increasing cell growth would not be motivated to combine the teachings of the Dinbergs and WO 94/23740 or WO 96/27657, because these references emphasizes increasing cell growth, in direct contrast to the present invention. Moreover, a combination of Dinbergs and WO

94/23740 or WO 96/27657 would change the basic principle under which each was designed to operate. As described above, WO 94/23740 and WO 96/27657 were designed to stimulate cell growth and proliferation. But according to Dinbergs, exposure to TGF-β would actually inhibit cellular proliferation, preventing cell growth. Therefore, there would be no motivation to combine the teachings of Dinbergs and WO 94/23740 or WO 96/27657. The proposed '430 Patent-Dinbergs-WO 94/23740 or -WO 96/27657 combination has no reasonable expectation of successfully achieving the invention. Not only does the above-described incompatibility of the '430 Patent and Dinbergs with WO 94/23740 or WO 96/27657 preclude a suggestion or motivation to combine, the combined teachings of these references fail to provide an ordinary artisan with any, much less a reasonable, expectation of successfully making the invention. As discussed above, Dinbergs teaches that the use of proliferation-inhibiting compounds in the '430 Patent and WO 94/23740 or WO 96/27657 would not succeed in stimulating tissue growth, because exposure to these compounds would actually inhibit cellular proliferation. Because the teachings of the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 are incompatable.

Appellants' arguments have been fully considered but are not found to be persuasive.

In response to appellants argument that there is no motivation to combine the teachings of Dinbergs and WO 94/23740 or WO 96/27657, because these references emphasizes increasing cell growth, rather than inhibition of growth, the strongest rationale for combining references is a recognition in the art that some advantage or expected beneficial result would have been produced by their combination. This recognition may be an expressed statement in a reference, an implication that can be drawn from one or more references or a convincing line or reasoning based upon established principles or legal precedent. In this case, The '430 provide clear direction, motivation and expectation of success in making a tissue engineering scaffold by covalently coupling to TGF-β to collagen matrix via a PEG polymer tether and the advantage of tethering factor molecules to the scaffold is that the effective amount of factor required is substantially reduced as taught by the '430 patent (see col. 7, lines 5-10, in particular). Dinbergs et al teach at concentration as low as 2 to 4 ng/ml, TGFβ inhibits the proliferation of smooth muscle cells, and endothelial cells. The WO 94/23740 publication teaches a method of inhibiting cell proliferation, see reference page 2, lines 14-15, stimulates bone formation, see reference page 4, lines 5-6, by stimulates matrix production such as bone deposition, see page 6, lines 13-15.

The WO 96/27657 publication teaches a method of making an engineering scaffold having any cell growth factor tethered to the scaffold, see entire document. The WO 96/27657 publication teaches the advantages of immobilization of tissue factor such as preventing receptor internalization upon ligand binding and considerably less growth factor is required because cells do not internalize and degrade the growth factor, see page 5, lines 13-30, in particular). The WO 96/27657 publication concludes immobilized growth factor on flexible tethers allow the ligand receptor complex to aggregate in the cell membrane (see page 6, lines 6-8, abstract, in particular). The very same advantages stated in the instant specification at 3, lines 20-23.

At page 42 of the Brief, even if the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 could properly be combined, such a combination would fail to obviate claim 1. None of the references alone or in combination discloses enhancing extracellular matrix formation without increasing cellular proliferation, or the benefit of doing so. Indeed, at an even more basic level neither reference teaches or suggests "covalently coupling the matrix-enhancing molecules to the scaffold in an effective density to elicit production of extracellular matrix without increasing cellular proliferation," as recited in independent claim 1. As discussed above, the '430 Patent and Dinbergs has not been shown to teach this limitation. Nor has either WO 94/23740 or WO 96/27657 been shown to supply this missing limitation. Nowhere has WO 94/23740 or WO 96/27657 been shown to discuss suitable concentrations TGF-β matrix-enhancing molecules, much less "an effective density to elicit production of extracellular matrix without increasing cellular proliferation." Accordingly, even if the cited references are combined, the Examiner has failed to show how the combination obviates claim 1. As claim 1 is nonobvious over the prior art, dependent claims 7 and 8 are similarly nonobvious. The combination of the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 fails to obviate the additional limitations found in dependent claim 8. At the outset Applicants note that in rejecting claim 8, the Examiner failed to show how the combination of the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 obviates claim 7, from which claim 8 depends. For this reason alone claim 8 has not been shown to be obvious over the combined references. Nevertheless, Applicants explain below that the combined references fail to teach or suggest the additional limitations present in claims 7 and 8.

Appellants' arguments have been fully considered but are not found to be persuasive. It is noted that the time period for inducing extracellular matrix production (over a 7-days time

period in culture, see specification 13, line 19-29, in particular) and cell proliferation (over 2-days, see page 14, lines 17) is inconsistent.

Dinbergs et al teach over a 2-days time period, the TGF-β concentration at 0.4 ng/bead or 2 to 4 ng/ml inhibits smooth muscle cell proliferation. The specification at page 14 discloses adding TGF-β to the media at 0, 1 or 5 ng/ml over a 2-day time period, ECM protein production was increased when TGF-β was added to the media at both 1 and 5 ng/ml. The cell numbers of smooth muscle cells did not increase over the 2 days, see page 14, lines 8-18. TGF-β is known to increase production of extracellular matrix protein by vascular smooth muscle cells growing in culture or in vivo (see page 6, lines 12-25 of the specification). Because the concentration of TGF-β of the reference is within the claimed range, the method of the combined teachings obviously have the same effects such as stimulates extracellular matrix production by the same smooth muscle cell type given that the type of matrix was not recited in the claim and the time at which the ECM was produced.

With respect to the argument that no where WO 94/23740 or WO 96/27657 have been shown to discuss suitable concentrations TGF-β matrix-enhancing molecules, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. In re Keller, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); In re Merck & Co., Inc., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). See MPEP 2145. The rejection is based on a combination of the US Pat 5,162,430 in view of Dinbergs *et al* and WO 94/23740 or WO 96/27657. The essence of the claimed invention is the method of making TGF-β tethered to biodegradable polymer scaffold and using the tethered TGF-β at a concentration between 1 and 5 ng/ml for *inhibiting* smooth muscle cell proliferation since increased proliferation of SMCs could lead to narrowing of a vessel lumen, see specification at page 3, lines 16-22. Further, TGF-β is known to increase production of extracellular matrix protein by vascular smooth muscle cells growing in culture or in vivo (see page 6, lines 12-25 of the specification). Dinbergs specifically discloses microspheres by adding TGF-β at a concentration of 3 ng TGF-β per microsphere. See Dinbergs at 29823, col. 2, third full paragraph. And each microsphere releases 0.4 ng TGF-β. See 29825, col. 1, second full paragraph ("one EVAc-BSA-TGF-J31 microsphere releases 0.4 ng TGF-β"); see also Dinbergs at 29823, col. 1, fifth full paragraph-29823, col. 2 ("Cell Proliferation Assay"). Given that three ng TGF-β per microsphere, 5-10 spheres were placed in 1 ml of PBS is equivalent to 15-30 ng/ml. Each

microsphere released 0.4 ng TGF-β, the minimum TGF-β released by 5-10 microsphere is in the range of 2 to 4 ng/ml, which is still within the claimed range of 1-100 ng/ml. In fact, the cell numbers of smooth muscle cells did not increase over the 2 days, see specification at page 14, lines 8-18.

At pages 42-43 of the brief, appellants argue that neither of WO 94/23740 and WO 96/27657 teaches or suggests the additional limitations of the scaffold being a hydrogel (claim 7), the hydrogel being formed of a polymer selected from the group consisting of alginate, collagen, hyaluronic acid, and polyethylene glycol polymers (claim 8). As discussed above, the polymers disclosed in WO 94/23740 are not themselves scaffolds, nor does WO 94/23740 even disclose tethering its polymer-conjugated growth factors to a scaffold. In WO 94/23740, the word "scaffold" and "hydrogel" are not even mentioned. Thus, even if the references are combined, the Examiner has failed to show that the combination obviates the additional limitations found in claims 7 and 8. Although WO 96/27657 discloses "attachment substrates," the Examiner has not shown how WO 96/27657 obviates claims 7 and 8. First, WO 96/27657 makes no mention of a scaffold formed from a hydrogel. The embodiment that the Examiner cites as disclosing "a scaffold such as hyaluronic acid (see page 7, line 1, in particular)" actually discloses a tether, rather than a scaffold. See WO 96/27657 at p 6, 1.27-p. 7, 1.2 ("Examples of water-soluble, biocompatible polymers which can serve as tethers include...."). And the embodiment that the Examiner cites as disclosing "polyethylene oxide, or alginate, (See page 17, line 8, in particular)" does not teach or suggest that these polymers are used in the form of a hydrogel. See WO 96/27657 at p 9, 11. 8-24. Indeed, WO 96/27657 notes that the attachment substrates may have various forms, but fails to even suggest a hydrogel. See WO 96/27657 at p 9, 11.25-29. Thus, even if the references are combined, the Examiner has failed to show that the combination obviates the additional limitations found in claims 7 and 8. Moreover, the proposed combination of the '430 Patent, Dinbergs, and WO 94/23740 or WO 96/27657 is improper because the Examiner has not provided a sufficient teaching, suggestion, or motivation in the prior art to make such a combination, as discussed above. Such improper combination cannot properly be used to obviate the subject claims.

Appellants' arguments have been fully considered but are not found to be persuasive.

The specification at page 5 lines 14-18 discloses hydrogel forming material is a particularly preferred hydrogel forming material is a polyethylene glycol-diacrylate polymer, which is photopolymerized. *Other hydrogel materials include* calcium alginate and certain other polymers that can form ionic hydrogels that are malleable and can be used to encapsulate cells. The specification discloses that there are a number of biocompatible polymers, both degradable and non-degradable. Representative synthetic non-biodegradable polymers include ethylene vinyl acetate and poly(meth)acrylate. Representative biodegradable polymers include polyhydroxyacids such as *polylactic acid* and polyglycolic acid, polyanhydrides, polyorthoesters, and copolymers thereof. Natural polymers include collagen, hyaluronic acid, and albumin, at page 5, lines 8-13.

The WO 94/23740 teaches hydrophilic polymer is a synthetic or natural polymer having render the polymer essentially water soluble, forms hydrogen bonds in aqueous solution such as *polylactic acid*, polyethylene glycol and derivative thereof, see page 5, lines 8-25, in particular and malleable by injection (see page 14, line 32-37, in particular).

The WO 96/27657 publication teaches various scaffolds such as biocompatible polymer such as polyglycolic acid, polyhydroxy acids, such as polylactic acid, collagen, polycosaminoglycans, collagens, alginate, hydroxyapatite and combination thereof (see page 9, lines 8-24, page 17, lines 1-12, in particular) that can be tethered by photocrosslinkerable agents (see page 12 line 18, in particular) to various growth factors such as the ones discloses at page 10, lines 20-29, in particular) for encapsulated cells (see page 16, line 12, in particular). The WO 96/27657 publication teaches the desire effect is dependent on the amount of immobilized factor (see page 16, lines 1-2). The WO 96/27657 publication teaches the reference malleable growth factor tethered scaffold can be administered to animals by various routes, such as implantation, injection and infusion (see page 18, lines 16-21, in particular).

Claims 24-27 and 32-34 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of US Pat No. 5,935,849 (of record, Aug 10, 1999; PTO 892).

At page 44-45 of the Brief, Appellants argue that the combination of the '430 Patent and the '849 Patent fails to obviate claims 24-27 and 32-34. First, the Examiner has improperly construed the scope of claim 24 as explained in detail above. Second, there is no reason, suggestion, or motivation to combine the references in the manner required to produce the

claimed invention. Third, even if the references are combined in the manner indicated by the Examiner, the resulting method would not include every limitation recited in Applicants' independent claim 24. The Examiner has improperly rejected claims 24-27 and 32-34 as obvious in view of art that clearly does not teach or suggest the claimed method of coupling a matrix-enhancing molecule to a tissue engineering scaffold in an effective density to increase extracellular matrix production without increasing cellular proliferation. In addition, the Examiner has not shown that the '430 Patent-'849 Patent combination teaches or suggests each and every limitation recited in Applicants' independent claim 24. The '849 patent fails to disclose tethers, matrix-enhancing molecules coupled to a scaffold, or suitable concentrations of matrix-enhancing molecules. The '849 Patent fails to disclose "covalently coupling the polymer tether to the scaffold," as recited by claim 24. The '849 Patent fails to disclose "covalently coupling the matrix-enhancing molecule to the scaffold," as recited by claim 24. The Examiner contends that the '849 Patent teaches covalently coupling to an inner matrix by a tether such as poly-d-lysine (see col. 18, line 30-35, in particular) coupling to matrix enhancing molecules such as RGD containing sequence (see col. 18, lines 36-51, in particular) or TGF beta and/or ascorbic acid (see col. 12, lines 56-67, in particular). (Final Office Action at 16.) The Examiner has mischaracterized the '849 Patent's teachings. First, RGD containing sequences are not matrix-enhancing molecules. Rather, these sequences are used to promote cellular attachment (see Application at 1, line 21-28). '849 Patent col. 18, 11.36-45. This mistake demonstrates how the Examiner's misconstruction of "matrix-enhancing molecules" has resulted in improper rejections. Second, the '849 Patent does not teach or suggest covalently coupling the matrix-enhancing molecules. The '849 patent fails to disclose "a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cells".

Appellants' arguments have been fully considered but are not found to be persuasive. With respect to the argument that the '849 patent fails to disclose the matrix-enhancing "covalently coupling the matrix-enhancing molecule to the scaffold", had the '849 patent also teaches this element, this rejection would have been rejected under 35 U.S.C 102(b) instead of under 35 U.S.C 103(a). However, the '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see

PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular) or insulin like growth factor or combination thereof (see col. 6, line 63, in particular). The reference polyethylene glycol has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The reference tissue engineering is useful for tissue or organ implantation or tissue regeneration (see col. 4, line 28-40, in particular). In fact, the specification discloses TGF-β was conjugated to polyethylene glycol (PEG) by reacting TGF-β with acryloyl-PEG-N-hydroxysuccinimide (acryloyl-PEG-NHS, 3400 Da), see instant specification at page 9, line 24-25.

With respect to the argument that RGD containing sequences are not matrix-enhancing molecules, the '849 patent teaches a method of making a tissue engineering scaffold such as bioartificial organ (BAO) using scaffold such as hydrogel or alginate or collagen (see col. 17, lines 66 through col. 18, col. 19, lines 22, col. 20, lines 42-36, summary of invention, in particular) where the luminal surface is tether to a polymer such as poly-d-lysine (see col. 18, line 30-35, in particular) and then coupling to matrix enhancing molecule such as *TGF beta and/or ascorbic acid* (see col. 12, line 56-67, in particular). The '849 patent teaches TGF beta is useful for inducing differentiation of fibroblast cells (concomitant inhibition of cell proliferation), and also as a growth inhibitor of keratinocytes and endothelial cells while ascorbic acid and TGFbeta1 increase extracellular matrix such as collagen biosynthesis (see col. 12, lines 57-67, Table 1, in particular). The reference method further comprises providing cells such as fibroblast or endothelial cells attached within the tissue-engineering scaffold (see col. 16, Table 1, Col. 19, line 29, in particular). The reference method is useful for implantation and controlling distribution of cells within the bioartificial organ (see claims of '849 patent).

With respect to the argument that the '849 patent fails to disclose tether, the '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer **tether such as hydrophilic polymer polyethylene glycol** (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGF-β (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular) or insulin like growth factor or combination thereof (see col. 6, line 63, in particular). Further, claim 24 does not recite a particular tether.

However, The '430 patent teaches polyethylene glycol tether has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). In fact, the '430 patent teaches the advantage of tethering factor molecules to the implant is that the effective amount of factor required is substantially reduced (see col. 7, lines 5-10, in particular). The '430 patent teaches the density or concentration of TGF-β ranges from about 1μg/ml to about 5mg/ml (see col. 10, lines 65-67, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired is within the purview of one ordinary skill in the art, these parameters may easily be determined by routine experimentation (see col. 13, lines 9-17, in particular). The '849 patent was cited for the teachings of including cells such as the ones recited in claim 27 within the scaffold.

With respect to the argument that the '849 patent fails to disclose "a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cells", it is noted that the particular concentration as required by certain cell type for the certain desired effect over a specific period of time are not recited in claim 20. The '430 patent teaches the advantage of tethering factor molecules to the implant is that the effective amount of factor required is substantially reduced (see col. 7, lines 5-10, in particular). The '430 patent teaches the density or concentration of TGF-β ranges from about 1μg/ml to about 5mg/ml (see col. 10, lines 65-67, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired is within the purview of one ordinary skill in the art, these parameters may easily be determined by routine experimentation (see col. 13, lines 9-17, in particular). In fact, the effect of increasing extracellular production by TGF is known in the art as early as 1991, see specification at page 6, lines 12-25. Further, the concept of tethering growth factor such as TGF-β to a tissue engineering matrix via a polymeric tether is also known in the art as evidenced by the teaching of WO 96/27657 publication of record, and specification at page 2, the lines 4-6. The reference concentration obviously has the same desired effects as claimed because the specific concentration of growth factor tethered to the scaffold is not recited in the claim. Since the Patent Office does not have the facilities for examining and comparing the claimed method to those of the prior art, the burden is on applicant to show that the prior art method is different from the claimed method. See In re Best. 562 F.2d 1252, 195 USPQ 430(CCPA 1977).

At page 46 of the Brief, Appellants argue that there is no suggestion in either the '430 Patent or '849 Patent to incorporate the teachings of the other. The '430 Patent and '849 Patent are incompatible and cannot be combined. As shown above, the '430 Patent teaches away from the claimed invention because it concerns cell proliferation. Because they teach different outcomes, the '430 Patent and '849 Patent are incompatible and cannot be combined.
The '849 Patent teaches using chemical compounds or growth factors that inhibit cell proliferation or induce differentiation to control cell distribution within a BAO. (See supra Section VI.B.1.) In contrast, the '430 Patent teaches cellular proliferation. (See supra Section V.A.) Because one reference teaches the inhibition of cell growth, while the other reference teaches the promotion of cell growth, the '430 Patent and '849 Patent are incompatible and cannot be combined. (See supra Section VI.B.) In light of these teachings, a combination of the '430 Patent and the '849 Patent would change the basic principle under which each of the '430 Patent was designed to operate. The '430 Patent was designed for sustained release of growth factors, such as TGF-J3, to "stimulate tissue growth to a detectable degree." '430 Patent, col. 7, 11. 18-22; col. 19, 11.10-56 and Figure 2. But according to the '849 Patent, exposure to TGF-β would actually inhibit cellular proliferation, preventing tissue growth to a detectable degree. Therefore, there would be no motivation to combine the teachings of the '430 Patent and the '849 Patent. The proposed '430 Patent-'849 Patent combination has no reasonable expectation of successfully achieving the invention. Not only does the above-described incompatibility of the '430 Patent with the '849 Patent preclude a suggestion or motivation to combine, that the combined teachings of these references fail to provide an ordinary artisan with any, much less a reasonable, expectation of successfully making the invention. As discussed above, the '849 Patent teaches that the use of proliferation-inhibiting compounds in the '430 Patent would not succeed in stimulating tissue growth, because exposure to these compounds would actually inhibit cellular proliferation, preventing tissue growth to a detectable degree. And the '430 Patent teaches that cellular proliferation is what is needed to stimulate tissue growth to a detectable degree. Because the teachings of the '430 Patent and the '849 Patent are incompatible, a skilled artisan would have no reason to expect that the proposed '430 Patent-'849 Patent combination would be successful to elicit production of extracellular matrix without increasing cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive.

In response to appellants' argument that the '430 Patent teaches cell proliferation rather than inhibition of cell proliferation, the '849 patent teaches TGF-β may be used to arrest or inhibit cell proliferation or to induce cell differentiation, see col. 12, lines 57-67, col. 13, line 1-2, in particular). The '849 patent further teaches immortalized cells or continuously proliferating cells can be operatively linked to a regulatable promorter to reduce or halted cell proliferation (see col. 10, lines 25-34, in particular). In response to applicant's arguments that there is no suggestion to combine the references, the examiner recognizes that obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. See In re Fine 5 USPQ2d 1596 (Fed. Cir 1988) and In re Jones 21 USPQ2d 1941 (Fed. Cir. 1992). In this case the teachings of the '849 patent pertaining to encapsulating cells in engineered scaffold and the use of any means such as TGF-β to arrest or inhibit cell proliferation and producing the desired effect and the teachings of the '430 patent indicating the success in tethering TGF-β to the collagen scaffold so that the effective amount of TGF-β is substantially reduced would have led one of ordinary skill in the art at the time the invention was made to combine the references to solve a well known problem in the art. The strongest rationale for combining reference is a recognition, expressly or implicitly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent that some advantage or expected beneficial result would have been produced by their combination In re Sernaker 17 USPQ 1, 5-6 (Fed. Cir. 1983), see MPEP 2144

At page 47 of the Brief, appellants argue that even if the '430 Patent and '849 Patent are combined, the combination fails to obviate claim 24 because neither reference alone, or in combination, discloses enhancing extracellular matrix formation without increasing cellular proliferation, or the benefit of doing so. Indeed, at an even more basic level neither reference teaches or suggests "covalently coupling the matrix-enhancing molecule to the scaffold, wherein the matrix-enhancing molecule is present at a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell," as recited in independent claim 24. As discussed above, the '430 Patent has not been shown to teach this limitation. Nor has the '849 Patent been shown to supply this missing limitation. Nowhere has the '849 Patent been shown to discuss suitable

concentrations of matrix-enhancing molecules, much less "a concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell." Accordingly, even if the cited references are combined, the Examiner has failed to show how the combination obviates claim 24. As claim 24 is nonobvious over the prior art, dependent claims 25-27 and 32-34 are similarly nonobvious.

Appellants' arguments have been fully considered but are not found to be persuasive. In response to appellants' argument that the '849 patent does not teach the suitable concentrations of matrix-enhancing molecules, the '430 patent teaches the concentration of TGF-β such as about 1μg/ml to about 5 mg/ml (see col. 10, lines 65-67, in particular). The '430 patent further teaches the amount of factor incorporated may easily be determined by routine experimentation, see col. 13, lines 9-17, in particular). Further, the particular concentration of matrix-enhancing molecule tethered to the scaffold for achieving inhibition of cell proliferation over a specific period of time is not recited in the claim 24. Likewise, the particular concentration of matrix-enhancing molecule tethered to the scaffold for achieving extracellular matrix production by the particular cell type over a specific period of time is not recited in claim 24. Further, the only concentration discloses in the specification is for TGF-β tethered to polymer. The optimal concentration to induce ECM production is in the range of between 1 and 5 ng TGF-β/ml for aortic smooth muscle cells over a 7 days time period and between 5 and 100 ng TGF-β/ml over a 7 days time period for auricular chondrocytes, see page 7, lines 17-20. With respect to ascorbic acid, EMB protein production was increased in the presence of 50 μg/ml soluble ascorbic acid added to the media and cell number did not increase over a 2 days period, see page 15. Again, the '849 Patent was cited for the teachings that TGF-β may be used for inhibiting cell proliferation, see col. 8, lines 62-67, col. 9, lines 1-2, in particular). In fact, TGF-β is an autocrine growth-inhibitory factor for cells such as human pancreatic carcinoid cells (see col. 13, line 6-8, in particular), Swhwamn cell (see col. 16, line 9, in particular). Finally, it has been held that where the general conditions of a claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. In re Aller, 220 F2d 454,456,105 USPQ233; 235 (CCPA 1955). See MPEP § 2144.05 part IIA.

Claims 27 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of US Pat No 5,935,849 (of record,

Aug 10, 1999; PTO 892) as applied to claims 24-27 and 32-34 mentioned above and further in view of Dinbergs *et al* (of record, J Biol Chem 271(47): 29822-29, 1996; PTO 892).

At bottom of page 47 of the Brief, appellants argue that the none of the references teach smooth muscle cell attached to the scaffold.

Appellants' arguments have been fully considered but are not found to be persuasive. With respect to argument that none of the references teach smooth muscle cell attached to the scaffold, it is noted that claim 27 recites a Markush group. The cells attached to the tissue engineering scaffold could be smooth muscle cells, endothelial cells, fibroblast, chondrocytes, *or* a combination thereof. The '849 patent teaches cells that can be attached to the tissue engineering scaffold could be any one of the cells listed in Table 1, see col. 16, in particular), any cells such as fibroblast (see col. 5, line 64, in particular), any muscle cell (myoblast), see col. 6, line 29-48, in particular), endothelial cells (see col. 16, line 37, in particular). Dinbergs et al teach smooth muscle cell and endothelial cells, see reference FIG. 3A-B, page 29825.

At page 47-48 of the Brief, appellants with respect to claim 29, Applicants note that none of the references teach the matrix-enhancing molecule being TGF-β coupled to the scaffold at the claimed concentration. As Applicants have previously pointed out, Dinbergs actually discloses a concentration for TGF-β far below what Applicants have claimed. Accordingly, even if the references are combined, the Examiner has failed to show that the cited combination obviates the additional limitations found in claim 29. Moreover, the proposed combination of the '430 Patent, the '849 Patent, and Dinbergs is improper because the Examiner has not provided a sufficient teaching, suggestion, or motivation in the prior art to make such a combination.

Appellants' arguments have been fully considered but are not found to be persuasive. As discussed above, the 430 patent teaches matrix-enhancing molecule being TGF-β coupled to the scaffold. The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFbeta (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular) or insulin like growth

factor or combination thereof (see col. 6, line 63, in particular). The '430 patent teaches the advantage of tethering factor molecules to the implant is that the effective amount of factor required is substantially reduced (see col. 7, lines 5-10, in particular). The '430 patent teaches the density or concentration of TGF-β ranges from about 1μg/ml to about 5mg/ml (see col. 10, lines 65-67, in particular). The '430 patent further teaches the amount of growth factor incorporated in the composition, the rate of delivery desired is within the purview of one ordinary skill in the art, these parameters may easily be determined by routine experimentation (see col. 13, lines 9-17, in particular). In fact, the effect of increasing extracellular production by TGF is known in the art as early as 1991, see specification at page 6, lines 12-25. Further, the concept of tethering growth factor such as TGF-β to a tissue engineering matrix via a polymeric tether is also known in the art as evidenced by the teaching of WO 96/27657 publication of record, and specification at page 2, the lines 4-6.

The claimed invention in claim 29 differs from the combined teachings of the references only in that the TGF-β is present at a density between 1 and 100 ng TGFβ/ml.

Dinbergs *et al* teach a method for making a tissue engineering scaffold such as alginate/heparin-sepharose microsphere for inducing formation of extracellular matrix by cells such as endothelial cells and smooth muscle cells bound to said scaffold comprising coupling various matrix-enhancing molecule such as bFGF or TGFβ in a concentration 1-10 ng/ml (See Alginate/Heparin-Sepharose Microsphere Preparation and Growth Factor Incorporation, page 29823, column 2, bridging page 29824 column 1, in particular). Dinbergs specifically discloses microspheres by adding TGF-β at a concentration of 3 ng TGF-β per microsphere. See Dinbergs at 29823, col. 2, third full paragraph. Each microsphere releases 0.4 ng TGF-β. See 29825, col. 1, second full paragraph ("one EVAc-BSA-TGF-β microsphere releases 0.4 ng TGF-β"); see also Dinbergs at 29823, col. 1, fifth full paragraph-29823, col. 2 ("Cell Proliferation Assay"). Given that three ng TGF-β per microsphere, 5-10 spheres were placed in 1 ml of PBS is equivalent to 15-30 ng/ml. Each microsphere released 0.4 ng TGF-β, the minimum TGF-β released by 5-10 microsphere is in the range of 2 to 4 ng/ml, which is still within the claimed range of 1-100 ng/ml. Further, the cell numbers of smooth muscle cells did not increase over a 2-days time period, see specification at page 14, lines 8-18. The reference TGFβ is known to elicit production of extracellular matrix (see page 29822, column 2, last paragraph, in particular) without increasing cellular proliferation (See Fig 2B, Fig 3B, Abstract, in particular). Dinbergs *et al* teach TGFβ has been incorporated into scaffold or various biodegradable polymer matrix such as

collagen, hydrogel such as alginate, hydron (hyaluronic acid) and polyethylene glycol polymers (See page 29827, column 2, first full paragraph, in particular). Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to optimize the concentration of TGFbeta such as the concentration as taught by Dinbergs *et al* that is known to inhibits cell proliferation as taught by the '849 patent and Dinbergs et al and then coupled the TGF-β at the optimized concentration for the particular cell type to the scaffold as taught by the '430 patent. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Dinbergs *et al* teach TGFβ is useful for eliciting extracellular matrix formation without increasing cellular proliferation for up to five days when coupling to various polymer such as alginate hydrogel for a sustained release (See page 29825, Fig 3A, in particular). The method of tissue engineering is useful for tissue or organ implantation or tissue regeneration as taught by the '430 patent (see col. 4, line 28-40, in particular).

In response to applicant's argument that there is no suggestion to combine the references, the examiner recognizes that references cannot be arbitrarily combined and that there must be some reason why one skilled in the art would be motivated to make the proposed combination of primary and secondary references. In re Nomiya, 184 USPQ 607 (CPA 1975). However, there is no requirement that a motivation to make the modification be expressly articulated. The test for combining references is what the combination of disclosures taken as a whole would suggest to one of ordinary skill in the art. In re McLaughlin, 170 USPQ 209 (CCPA 1971). References are evaluated by what they suggest to one versed in the art, rather than by their specific disclosures. In re Bozek, 163 USPQ 545 (CCPA 1969).

Claims 24 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of Scott-Burden et al (of record, J Cardiovasc Pharmacol 16 Suppl 4: S36-41, 1990; PTO 892).

At pages 48-49 of the Brief, Appellants argue that neither the '430 Patent nor Scott-Burden suggest that angiotensin II would elicit extracellular matrix production without increasing cellular proliferation. As shown above, the '430 Patent concerns cell proliferation. Thus, growth factors, such as insulin-like growth factor and TGF-J3, may be used at concentrations sufficient "to stimulate tissue growth to a detectable degree." '430 Patent, col. 6, 11.58-63, col., 7, 11. 19-20. Scott-Burden teaches that angiotensin II is "capable of stimulating both growth and matrix elaboration by cultured vascular smooth muscle cells." Scott-Burden at $36. Maintenance of vascular smooth muscle cells in their proliferative form for a prolonged period leads to vascular thickening and vessel occlusion. Scott-Burden at $36. Therefore, Scott-Burden sought to understand how the vasoconstrictor angiotensin II impacts both cellular proliferation and the modulation of extracellular matrix. Scott-Burden at S37. Their findings demonstrate that angiotensin II stimulates both cellular proliferation and production of extracellular matrix. Scott-Burden at $36, $40; (see also, Final Office Action at 11 ("Scott-Burden et al teach angiotensin II activates the synthesis of extracellular matrix such as glycopeptides and proteoglycans by smooth muscle cells and growth of smooth muscle cell (see abstract, in particular)." (emphasis added)). In light of these teachings, the '430 Patent-Scott-Burden combination would change the basic principle under which each reference was designed to operate. Namely, the teachings of Scott-Burden suggest that the substitution of angiotensin II for TGF-13 in the invention of the '430 Patent would result in increased cellular proliferation along with increased production of extracellular matrix. Neither reference discloses or suggests that it is desirable or possible to use angiotensin II, in substitution for TGF-13, to result in production of extracellular matrix without a concomitant increase in cellular proliferation (as required by Applicants' independent claim 24 and dependent claim 30). Therefore, these prior art references fail to provide a reason why one of ordinary skill in the art would have been motivated to combine the teachings of the '430 Patent and Scott-Burden, much less how such a combination would arrive at the claimed invention. Furthermore, the nature of the problem solved by the claimed invention is to stimulate production of extracellular matrix without additionally stimulating cellular proliferation. When each cited reference is considered as a whole, both disclose use of biologically active factors to stimulate tissue growth. Therefore, it would not have been desirable, and thus not obvious, to one of ordinary skill in the art at the time of the invention was made to combine the teachings of the '430 Patent and Scott-Burden in order to address how to stimulate extracellular matrix production

without a concomitant increase in cellular proliferation. Therefore, the combination of the '430 Patent and Scott-Burden is improper.

Appellants' arguments have been fully considered but are not found to be persuasive.

The '430 patent teaches a method of making a tissue engineering scaffold by providing a scaffold such as collagen (col. 4, lines 5-6, in particular) covalently coupled to a polymer tether such as hydrophilic polymer polyethylene glycol (see PEG, col. 5, lines 32-55, in particular) covalently coupled to a matrix enhancing molecule such as TGFβ (col. 6, line 58, see entire document, col. 8, General Method, Examples 1 and 6, col. 19, in particular). The reference polyethylene glycol has a molecular weight of between 1900, and about 8,000, which is between the claimed 200 and 10,000 (see col. 5, line 39, in particular). The advantage of covalently coupled to a matrix enhancing molecule (growth factor) to the scaffold is that substantially less effective amount of growth factor is required, see '430, col. 7, lines 5-10, in particular). Scott-Burden et al teach culture smooth muscle cells exposed to angiotensin II exhibit an enhanced synthesis of extacellular matrix production and is accompanied by increased rates of proliferation (see page S96, col. 2, in particular). Scott-Burden et al teach the problem of increasing smooth muscle cell proliferation is that this proliferative activities may lead to the structural changes associated with hypertension and atherosclerosis that in themselves further stimulate the proliferative behavior of smooth muscle cells, see page S96, paragraph bridging col. 1 and 2, in particular). In fact, the specification discloses increased proliferation of smooth muscle cells could lead to narrowing of a vessel lumen, at page 3, line 17-18.

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the TGFbeta tethered to the scaffold as taught by the '430 patent for the angiotensin II as taught by Scott-Burden et al and then optimize the concentration of the tethered angiotensin II for a method of for making a tissue engineering scaffold for inducing formation of extracellular matrix without cell proliferation. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do reduce smooth muscle cell proliferation because Scott-Burden et al teach the problem of increasing smooth muscle cell proliferation may lead to the structural changes associated with hypertension and atherosclerosis that in themselves further stimulate the proliferative behavior of smooth muscle

cells, see page S96, paragraph bridging col. 1 and 2, in particular). One having ordinary skill in the art would have been motivated to covalently coupled to a matrix enhancing molecule (growth factor) to the scaffold is that substantially less effective amount of growth factor is required, see '430, col. 7, lines 5-10, in particular) and the amount of factor incorporated may easily be determined by routine experimentations (see col. 13, lines 9-14, in particular).

At page 49-50 of the Brief, appellants argue that the proposed combination fails to teach a concentration of matrix enhancing molecule sufficient to elicit extracellular matrix production without increasing cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive. With respect to "concentration sufficient to elicit production of extracellular matrix by a cell attached to the matrix without increasing cellular proliferation of the attached cell", the instant specification does not teach the concentration of angiotensin II elicits extracellular production without increasing cellular production at any concentration, much less which cell type attached to the matrix such that the concentration is effective to inhibit the proliferation of such cell. The specification merely asserts that matrix enhancing molecule such as angiotensin II covalently coupled to any scaffold at a concentration that is effective to elicit production of extracellular matrix by any cell attached to the scaffold without increasing cellular proliferation of the attached cells for the claimed method. There is no evidence of record in the specification as filed demonstrating that angiotensin II elicits extracellular production without increasing cellular production at any concentration for any cell type. In fact, the specification discloses the concentration for the same growth factor such as TGF-β differs with respect to the type of cell attached to the scaffold. In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng TGF-β/ml for aortic smooth muscle cells and between 5 and 100 ng TGF-β/ml for auricular chondrocytes, which is equivalent to between 4×10^{-6} and 4×10^{-3} nmol/ml, see page 7, lines 11-20. For ascorbic acid, the concentration was 50 μg/ml and is not even tethered to any scaffold for increasing ECM production for both SMC and chondrocytes, see page 15 of specification. The specification discloses only the concentration for TGF-β and ascorbic acid. The specification does not disclose such concentration could be generalized to other matrix enhancing molecule for the claimed method and for any and all cell types.

Claims 24 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat 5,162,430 (of record, Nov 10, 1992; PTO 892) in view of WO 94/23740 (of record, Oct 1994, PTO 1449) or WO 96/27657 (of record, Sept 1996; PTO 1449).

At page 50-51 of the Brief, the '430 Patent and WO 94/23740 and WO 96/27657 both concern cell proliferation. In contrast, the present invention discloses, among other things, increasing production of extracellular matrix while minimizing cell growth. Accordingly, one of ordinary skill attempting to increase production of extracellular matrix without increasing cell growth would not be motivated to combine the teachings of the '430 Patent and WO 94/23740 or WO 96/27657, because these references emphasizes increasing cell growth, in direct contrast to the present invention. WO 94/23740 fails to disclose a method of making a tissue engineering scaffold, or that matrix enhancing molecules may be coupled to such a tissue engineering scaffold. As with claim 1, the Examiner has misinterpreted the WO 94/23740 disclosure, and WO 94/23740 does not disclose methods of making a tissue engineering scaffold--much less methods of making a tissue engineering scaffold involving coupling a matrix enhancing molecule to the scaffold, a limitation that is recited in Appellants' claim 24. WO 96/27657 does not disclose covalently coupling a matrix enhancing molecule to a scaffold to increase extracellular matrix production without increasing cellular proliferation. The Examiner has failed to show that WO 94/23740 discloses tethering a matrix enhancing molecule to a scaffold to increase extracellular matrix production without increasing cellular proliferation, as recited in Appellants' claim 24.

Appellants' arguments have been fully considered but are not found to be persuasive.

The WO 94/23740 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 covalently coupling to polyethylene glycol (See page 12, line 11, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication teaches the method of making a tissue engineering scaffold comprising coupling TGFβ to a polymer is useful for stimulation of bone formation at a lower dose (See abstract, in particular). With respect to the argument that the WO 94/23740 teaches emphasize cell growth and proliferation rather than inhibition of cell proliferation, the WO 94/23740 publication teaches a method of **inhibiting cell proliferation**, see reference page 2, lines 14-15, stimulates bone formation, see reference page 4, lines 5-6, by stimulates matrix production such as bone deposition, see page 6, lines 13-15.

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) covalently coupled to a scaffold such as hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate, (See page 17, line 8, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular). The WO 96/27657 publication teaches the use of cell growth substrates with tethered cell growth factor molecule to stimulate tissue replacement by **limiting the amount of tissue growth** and eliminating the need to remove the tissue scaffold once implanted (see page 9, lines 5-7, page 10, lines 3-10, in particular).

At page 52-53 of the Brief, Appellants argue that WO 94/23740 and WO 96/27657 concern cell proliferation, which teaches away from the claimed invention. (See also supra Section VII.B.) Indeed, cell growth is expressly stated to be an object of the WO 96/27657 invention. See WO 96/27657 at 3, 11.21-27. And "stimulating bone formation" is described in WO 94/23740. See, e.g., WO 94/23740 at 4, 11. 1-19; ld. at 20, I1.7-22 (noting significant increases in proliferation of osteoblast-like cells, which was interpreted as bone stimulation). Thus, WO 94/23740 and WO 96/27657 teach away from the present invention, which discloses, among other things, increasing production of extracellular matrix while minimizing cell growth. The inclusion of WO 94/23740 and WO 96/27657 in the obviousness rejections represents clear error that must be reversed. Neither reference discloses the coupling of matrix-enhancing molecules to a tissue engineering scaffold, nor discloses coupling matrix-enhancing molecules in an effective density to enhance extracellular matrix production without increasing cellular proliferation. Thus, the proposed combination fails to teach a concentration of matrix enhancing molecule sufficient to elicit extracellular matrix production without increasing cellular proliferation. Applicants' combination of immobilized growth factor technology and the inhibitory properties of TGF-β results in unexpectedly improved methods that involve enhancing extracellular matrix formation without an increase in cell proliferation. The cited prior art, as discussed above shows or describes increases in cellular proliferation when matrix-enhancing molecules, like TGF-β, are introduced to the cell population. For example: the '430 Patent concerns stimulating tissue growth to a detectable degree (col. 7, 11. 17-22); Dinbergs argues that TGF-β should not be released in a sustained manner because of its reduced efficacy in inhibiting

cell growth, citing an 18.0-fold increase in endothelial cell number over the original plating density and a 115.0-fold increase for smooth muscle cells (see p. 29825, col. 1, last paragraph, bridging col. 2); Scott-Burden showed angiotensin II could stimulate proliferation and ECM production (see s36, Abstract); WO 94/23740 notes significant increases in proliferation of osteoblast-like cells (see p. 20, 11. 7-22); and WO 96/27657 is directed to methods and compositions for stimulating eukaryotic cell growth (see abstract & claims 1, 13, 31).
In many tissue engineering applications it is important to avoid undesirable enhancement of cell growth. For example, in vascular tissue engineering, over-proliferation of the smooth muscle cells can lead to a failure of the tissue engineering construct due to luminal narrowing. Applicants have devised methods that involve increasing extracellular matrix production without increasing cellular proliferation, by coupling matrix-enhancing molecules to a scaffold in an effective density. This is unexpected in light of the prior art: the '430 Patent discloses tethering TGF-I3 for sustained administration (col. 12, 1.63-col. 13, 1. 1); Dinbergs teaches TGF-β sustain-released from microspheres is unable to effectively inhibit cell proliferation; the '849 Patent discloses replenishing growth factors, but does not disclose covalent coupling or increasing ECM (col. 13, 64-col. 14, 1. 6); Scott-Burden discloses angiotensin II's ability to stimulate proliferation and ECM production (see S36, Abstract); WO 94/23740 discloses a soluble polymer-conjugated growth factor that increases cellular proliferation; and WO 96/27657 discloses growth factors coupled to scaffolds that increase cellular proliferation.

Appellants' arguments have been fully considered but are not found to be persuasive.

With respect to the argument that the WO 94/23740 teaches emphasize cell growth and proliferation rather than inhibition of cell proliferation, the WO 94/23740 publication teaches a method of inhibiting cell proliferation, see reference page 2, lines 14-15, stimulates bone formation, see reference page 4, lines 5-6, by stimulates matrix production such as bone deposition, see page 6, lines 13-15. Likewise, the WO 96/27657 publication teaches the use of cell growth substrates with tethered cell growth factor molecule to stimulate tissue replacement by limiting the amount of tissue growth and eliminating the need to remove the tissue scaffold once implanted (see page 9, lines 5-7, page 10, lines 3-10, in particular).

With respect to the argument that the coupling matrix-enhancing molecules in an effective density to enhance extracellular production without increasing cellular proliferation, it is noted that the claims are not limited to any matrix enhancing molecule such as TGF, nor any cell type attached to the scaffold, nor any particular concentration.

With respect to the argument that neither reference discloses the coupling of matrix-enhancing molecules to a tissue engineering scaffold, the WO 94/23740 publication teaches a method for making a tissue engineering scaffold by coupling various matrix-enhancing molecules such as TGFβ or TGFβ2 covalently to polyethylene glycol via a tether or linking group such as hydroxysuccinimide or polymer-glutarate (See page 11, lines 10-35, page 12, line 11, PEG-TGF-β conjugates, rhTGF- TGF-β2 (PEG5000) bridging page 13, in particular). The WO 94/23740 publication teaches the method of making a tissue engineering scaffold comprising coupling TGFβ to a polymer is useful for stimulation of bone formation at a lower dose (See abstract, in particular). The bone formation is by stimulating bone deposition or extracellular production, see WO 94/23740 publication at page 6, line 12-15.

The WO 96/27657 publication teaches a method for making a tissue engineering scaffold comprising coupling various matrix-enhancing molecules such as TGFβ (see page 10, claim 25 of WO 96/27657 publication, in particular) covalently coupled to a scaffold such as hyaluronic acid (see page 7, line 1, in particular) or polyethylene oxide, or alginate, (See page 17, line 8, in particular). The WO 96/27657 publication teaches the growth factor is localized to desired target cell population and significantly less growth factor is needed to exert a biologic response (See abstract, in particular). The WO 96/27657 publication teaches the use of cell growth substrates with tethered cell growth factor molecule to stimulate tissue replacement by **limiting the amount** of tissue growth and eliminating the need to remove the tissue scaffold once implanted (see page 9, lines 5-7, page 10, lines 3-10, in particular).

With respect to combined teachings fail to teach the concentration of matrix enhancing molecule sufficient to elicit extracellular matrix production without increasing cellular proliferation, other than the concentration of TGF-β tethered to polymer, there is no evidence of record in the specification as filed showing other extracellular matrix such as angiotensin II elicits extracellular production without increasing cellular production at any concentration for any cell type. In fact, the specification discloses the concentration for the same growth factor such as TGF-β differs with respect to the type of cell attached to the scaffold. In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng TGF-β/ml for aortic smooth muscle cells and between 5 and 100 ng TGF-β/ml for auricular chondrocytes, which is equivalent to between 4×10^{-6} and 4×10^{-3} nmol/ml, see page 7, lines 11-20. For ascorbic acid, the concentration was 50 μg/ml and it is not even tethered to any scaffold

for increasing ECM production for both SMC and chondrocytes while inhibits cell proliferation over a 2-days time period, see page 15 of specification.

Appellants' argument of unexpected results is acknowledged. However, claims 24 and 27, 30-35 are not limited to TGF-β as argued. The evidence of record do not overcome clear and convincing evidence of obviousness. In fact, the specification discloses the concentration for the same growth factor such as TGF-β differs with respect to the type of cell attached to the scaffold. In the case of TGF-β, optimal concentrations to induce ECM production is in the range of between one and five ng TGF-β/ml for aortic smooth muscle cells and between 5 and 100 ng TGF-β/ml for auricular chondrocytes, which is equivalent to between 4×10^{-6} and 4×10^{-3} nmol/ml, see page 7, lines 11-20. For ascorbic acid, the concentration was 50 μg/ml and it is not even tethered to any scaffold for increasing ECM production for both SMC and chondrocytes while inhibits cell proliferation over a 2-days time period, see page 15 of specification. Further, the burden of proof is the same and its fairness is evidenced by the PTO's inability to test claimed method for making all other extracellular matrix enhancing molecule other than TGF-β tethered to any polymer that has the asserted effects for all cell type that attached to the scaffold and compare prior art method of making the same products. Examiner properly shifted burden to appellants to establish, through objective evidence, that method of invention differ in unobvious manner from those of the prior art references. Ex parte Phillips, 28 USPQ2d 1302 (BPAI 1993). Here, appellants have not provided any objective evidence to support the difference between the prior art and instant method of making all extracellular matrix enhancing molecule. The record does not contain sufficient objective evidence that the referenced method differ in any significant manner from that claimed. Even the claims are limited to TGF-β, the concentration for each type of cell differ. As such, there is insufficient evidence to conclude that the claimed concentration sufficient to elicit production of extracellular matrix by a cell attached to the tissue engineering scaffold without increasing cellular proliferation of the attached cell.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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